Haematological and histopathological studies on the effect of some heavy metals on Labeo rohita (Ham) and *Clarias batrachus* (Linn.)

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Dedicated

to

My Parents, Teachers

&

My Sister Sudha

CERTIFICATE

I am pleased to certify that the work incorporated in the thesis entitled " ***Haematological and histopathological studies on the effect of some heavy metals on Labeo rohita*** **(Ham) and** ***Clarias batrachus*** **(Linn.)"** submitted by **Mr. Sudhir Tiwari,M.Sc.** was carried out by the candidate under my supervision. He has put in over 200 days of attendance in the laboratory to complete the work.

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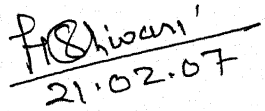

(Sudhir Kumar Tiwari)

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SUMMARY

SUMMARY

Due to rapid industrialization and urbanization, large amounts of heavy metals and their compounds are continuously releasing the reverine system of India in general and of the industrial areas province in particular. With the extensive growth of industrial development the use of metals have grown enormously. Metals play an important part in modem societies and have historically been linked with industrial development and improved living standards. Society can draw on metal resources from Earth's crust as well as from metal discarded after use in the economy.

Chapter – I deals with the introduction of the problem taken up for investigation, due to load of heavy metal in environment the aquatic as well as terrestrial animal get affected from it. The fish *Labeo rohita* and *Calrias batrachus* are very common fish inhabiting ponds and river such as Betwa and Pahuj River passing through district Jhansi. These fish form a major bulk of the edible variety of the fish locally. Hence in the present study the effect of heavy metals, cadmium, mercury and nickel has been studied as cadmium chloride, mercuric chloride and nickel sulphate.

Chapter – II deals with the historical account of the research work, which has been undertaken by different workers on the effect of different metals and other pollutants on fish.

Chapter – III, deals with the experimental programme which has been discussed, wherein material and methods used for the present investigations have been given. Three heavy metals cadmium, mercury and nickel have been used in the form of cadmium chloride, mercuric chloride and nickel sulphate. Two fresh water edible varieties of fish, *Calrias batrachus* and *Labeo rohita* have been used to determine the toxicity of these metallic pollutants. In these experiments *Clarias batrachus* is treated with cadmium chloride and nickel sulphate while

Labeo rohita is subjected to mercuric chloride. Observations were made for acute toxicity, causing mortalities among the fish used for test. Median lethal concentration values for different exposure duration, 24 hrs, 48 hrs, 72 hrs, and 96 hrs were ascertained. Accumulation of these metals in vital organs was also observed. The method for conducting these experiments followed EIFAC (1983)[50] and APHA (1985)[12]. Lethal threshold concentration for each metal and fish were recorded. Histological changes were studied. The change in weight and length due the effect of metal were studied.

Chapter – IV describes the following results observed in the experiment and Chapter V deal with discussion in details. For *Labeo rohita* the LC50 value was recorded on probit analysis is 680 mg/l, 533 mg/l, 516 mg/l and 428 mg/l for 24 hrs, 48 hrs, 72 hrs and 96 hrs. The lethal threshold concentration which is the minimum concentration o the metal, sufficient enough to cause first mortality within 96 hrs, was observed to be 50 mg/l and the minimum concentration at which 100 % mortality was recorded in 30 days exposure was 100 mg/l. Lethal threshold concentrating of cadmium chloride for fish *Clarias batrachus* found to be 0.5 mg/l in which the first mortality was recorded in 48 hrs. Median lethal concentration for cadmium sulphate were recorded as 4.6 mg/l, 2.85 mg/l, 2.62 mg/l and 2.31 mg/l for 24 hrs, 48 hrs, 72 hrs and 96 hrs durations. 6.0 mg/l concentration was found to be highly toxic which caused 100 % mortality in 48 hrs.

Nickel toxicity was observed in fish *Clarias batrachus*. Lethal threshold value of nickel is recorded as 1.0 mg/l in which first mortality was recorded in 96 hrs. LC50 value were calculated to be 11.36 mg/l, 8.8 mg/l, 7.25 mg/l and 6.6 mg/l for 24 hrs, 48 hrs, 72 hrs, and 96 hrs, At 12.0-mg/l concentrations, all the fish were found to be dead in 96 hrs.

Studies on the growth in length and weight of the fish were made. The maximum growth in length of *Labeo rohita* subjected to mercuric chloride was recorded to be 3.07 percent in 200-mg/l concentrations while

minimum growth in length is 2.85 Percent was observed in 300-mg/l concentrations. In weight the maximum growth was observed in 50-mg/l mercuric chloride while loss of weight by 0.87 percent was recorded in 300-mg/l concentrations. In the studies in *Clarias batrachus* subjected to cadmium chloride, the fish showed 12.5 and 6.25 percent growth in length in 0.5 mg/l and 1.0 mg/l concentration while in other concentration the growth was not observed. In weight the maximum growth of 5.862 percent was observed in 0.5-mg/l concentrations. A loss in weight was registered in 2.0 and 2.5 mg/l concentration.

The effect of nickel sulphate was studied on *Clarias batrachus* where maximum increase in length was found to be 11.76 percent in 1.0 mg/l and maximum gain in weight was recorded to be 0.291 percent in 1.0 mg/l nickel sulphate. While loss of weight, which was measured by 0.140 percent and 0.099 percent in 4.0 and 6.0 mg/l concentration. The histopathological studies were made to observed the effect of these metals on different organ of fish; these histopathological changes were observed in gill, liver and kidney of the fish. Due to the effect of metals, these organs showed lesions and different degrees of degenerative changes depending upon the dose i.e. concentration of the metals and the duration of the exposure. In gill due the effect of these metals the first symptom which are come is that the secretion of the mucous, degenerative changes in gills include, vacuolar degeneration of the cytoplasm of the epithelial cells, necrosis, haemorrhage, hyperplasic and hypertrophy. Histopahtological change occur in liver is degenerative of cytoplasm hepatocytes, necrosis, hyperemia, haemorrhage and engorgement of blood cell in the blood vessel. Tublonecrosis, degenerative changes in the epithelial cell of the tubule, haemorrhage and oedematos conditions of Bowman's capsule were the histopathological changes seen in the kidney of the fish. Histopathological changes is seen

more prominent in the fresh water fish " *Labeo rohita* due to the toxic effect of mercuric chloride as compare to fresh water fish *Clarias batrachus* due to the toxic effect of cadmium chloride and due to the nickel sulphate the lest effect is observed.

In gill, liver and kidney the accumulation of metals (cadmium, mercury and nickel) was also studied. The accumulation in gills, liver and kidney has been found to increase with concentration of the metal used and the duration of the exposure. In gill the minimum accumulation of cadmium is occur in 48 hrs is 3.6 percent in 0.5 mg/l concentration of cadmium chloride while maximum concentration is 12.8 percent was found to accumulate in 5 days. In liver the minimum accumulation of is 1.2 percent in same concentration of 0.5 mg/l. and maximum accumulation is 4.25 percent in 5.0 mg/l concentration of cadmium chloride. In kidney the minimum accumulation is 0.4 percent and maximum 2.3 percent of cadmium accumulations was observed.

Accumulation of 3.1 percent of mercury has occurred in 24 hrs in 50-mg/l concentration of mercuric chloride. Same as 5.2, 5.6, 5.7 and 6.0 percent of accumulation has occurred in gill at 24 hrs in 100 mg/l, 200 mg/l, 400mg/l and 500-mg/l concentration of mercuric chloride. Maximum accumulation of mercury are 25.0, 28.4, 31.0, 30.2 and 29.1 percent in 30, 30, 25, 20 and 20 days respectively.1.3 percent and 10.4 percent of mercury were accumulate in liver in 50 mg/l concentration of mercuric chloride in 24 hrs, and in 100 mg/l concentration in 24 hrs the value of accumulation were found to be is 3.4 and 11.7 percent. 13.2 percent is maximum accumulation of mercury in liver were found to be in 400 mg/l concentration of mercuric chloride in 20 days. In kidney the minimum accumulation of mercury is found to be 1.1 percent in the duration of 24 hrs and maximum accumulating is 7.6 percent in the duration of 20 days in 50 mg/l and 400-mg/l concentrations respectively.

In gill for nickel, minimum accumulation noted is 1.2 percent in 24 hrs in 1.0 mg/l concentration of nickel sulphate and maximum accumulation is 16.5 percent in 8.0-mg/l concentration of nickel sulpahte in 30 days. For the liver the value of minimum accumulation is 0.5 percent in 1.0 mg/l in 24 hrs and maximum accumulation is 4.5 percent in 8.0 mg/l in 30 days of duration. In kidney the accumulation is not be deductive in least concentration and duration. In 6.0-mg/l concentration of nickel sulphate the accumulation of nickel was found to be is 0.2 percent in 24 hrs and this is the minimum accumulation of nickel in kidney while the maximum accumulation of nickel is 2.6 percent in 8.0 percent in 30 days.

By present investigation it is clearly indicated by the observation and the results that of these three metals taken to study the effect on the fish, mercury is highly, cadmium is less and nickel is least toxic for the fish.

Introduction

Fish have great significance in the life of mankind, being an important natural source of protein and providing certain other useful products as well as economic sustenance to many nations. The gradual erosion of commercial fish stocks due to over -exploitation and alteration of the habitat is one reason why the science fish biology came into existence (Royce, 1972). It is a well known fact that the knowledge on fish biology particularly on morphometry, length-weight relationship, condition factor, reproduction, food and feeding habit, etc. is of utmost important not only to fill up the lacuna of our present day academic knowledge but also in the utility of the knowledge in increasing the technological efficiencies of the fishery entrepreneurs for evolving judicious pisciculture management. For developing fishery, it is necessary to understand their population dynamicshow fast they grow and reproduce, the size and age at which they spawn; their mortality rates and its causes, on what they prey upon along with other biological processes. There are many isolated disciplines in fish biology, of which the study of morphology is inseparably related to study of the mode of life of the organism. It fact, the size and shape are fundamental to the analysis of variation in living organisms (Grant and Spain, 1977) and morphological variations even in the same species most often related to the varied environmental factors.

In India, even though industrialization has not reached the level attained in the developed countries, pollution of aquatic habitats seems to be an inevitable problem. More toxic compounds are being increasingly detected in aquatic ecosystems. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated Khare, S., S. Singh (2002)[90]. Industrial discharges containing toxic and hazardous substances, including heavy metals, Gbem, T. T., J. K. Balogun, F. A. Lawal, P. A. Annune (2001); Woodling, J. D., S. F. Brinkman, B. J. Horn (2001).,contribute tremendously to the pollution of aquatic ecosystems. According to Satyanarayanan, D., I. M. Rao, B. R. Prashada Reddy

(1985)[167]. The presence of heavy metals on the east coast of India deserves special mention as it almost forms a repository for industrial effluents and city sewages. Among the various heavy metal pollutants, cadmium merits special attention due to its potential hazards to aquatic biota Mayer, W., M. Kretschmer, A. Hoffmann, G. Harish (1991)[111]; Barber, D., M. S. Sharma (1998)[18]., as well as to human beings Groten, J. P., P. J. Van Blanderen (1994)[68]; Vanderpool, A., G. Reeves (2001)[195]. This heavy metal is a common aquatic pollutant and is known to be highly toxic to most organisms, even at small concentrations in natural waters Lovert et al., (1972)[110]. In general, cadmium is a biologically non-essential, non-biodegradable, persistent type of heavy metal and its compounds are known to have high toxic potentials. Further, continuous, low-level cadmium exposure may have a gross biological impact comparable to that of recurring exposures of much greater intensity. In fresh water fish, cadmium uptake is taking place mainly through three routes namely, gills. On the other hand, the metal retention capacity of the fish is dependent on the metal assimilation and excretion capacities of the fish concerned Rao, L. M., R. Patnaik (1999)[153]. According to Ferard et al. (1983)[54]. aquatic organisms take up heavy metals and concentrate them to amounts considerably higher than those found in the environment. Therefore, it is important to find the pathways of accumulation of heavy metals and their affinity to different tissues, especially in fishes. In this context, the present investigation has been designed to study the pattern of bioaccumulation of cadmium in the gills, liver and kidneys of the culturable catfish *Clarias batrachus* (Bloch.) exposed to sublethal concentrations of cadmium chloride. *C. batrachus* is highly valued as a table fish throughout the Indian subcontinent and is preferred for culture even in muddy and shallow waters where other culturable fishes may not thrive well. . Obula Reddy KP et al. (1999)[131]. work on ambient ammonia effects on certain biochemical constituents have been studied in fry of *Cyprinus carpio*. Frys which are 30 days old were exposed to 2.3 ppm of liquor ammonia for 7,

14, 21 days. There was increment in total protein content in 7 days of ammonia exposed-fries while a decrement was observed in 14 and 21 days exposed fries. The possible reasons for these changes are discussed.

Pollutants affecting the natural environment include certain chemical elements released into ecosystems as a result of multifaceted activity of humans. Their presence changes individual development of both plants and animals. Biological effects of disturbed chemical homeostasis appear in the environment much earlier, before symptoms and biochemical changes appear in individual organisms. The fish are directly associated with water; they are an important component of human diet. For humans, they can be a source of xenobiotics that adversely affect human life functions Mudzki and Szkoda 1996. Sobecka (2001) proved disorders in the iron level in the organs and tissues of wells catfish, *Silurus glanis* L. caused by nickel. Sharma MS et all. (2000)[173] work on water temperature variations are found to influence senstivity of fresh water, zooplankton to heavy metal toxicants. Static short term bioassay exposing representative fresh water zooplankters to different concentrations of zinc, lead and cadmium revealed that sensitivity of Daphnia to metal and thermal stress was higher than cyclops and cypris. The most resistant planktonic animal against varying metal concentrations and different water temperatures was observed to be cypris. Prakash Ram et al. (1999)[140] says that it is essential to monitor the various health effecting parameters in the ground water before it is used for continuos long period by the people. It is observed that some trace elements viz. copper, manganese, molybdenun, lead, zinc, nickel and iron has been observed in the shallow ground water aquifer, which needs proper monitoring, awareness and removal before its use for drinking purposes. Ramudu K et al (2000)[147] study on adoptive changes in respiratory movements of an air-breathing fish, *Anabas testudineus* exposed to sublethal concentrations 1.9, 4.75 and 9.5 ppm for 21 days were studied. Significant increase ($P<0.001$) in surfacing

behaviour were observed in 1.9, 4.75 and 9.5 ppm monocrotophos treated fishes compared to control. Opercular movements decreased ($P<0.001$) in all three sublethal concentrations compared to control.

Large amounts of heavy metals and their compounds are continuously releasing the riverine system of India in general and of the industrial areas province in particular. These points towards desperate need for assessing the problem and to develop methods for alleviating the ill- effects of pollutants like lead and nickel because polluted water can cause paralysis, meningitis, cancer, sterility, schistosomiasis, poliomyelitis and filariasis in animals. Therefore, the present study was planned to assess the metals, viz. lead and nickel toxicities in fish and water of river Betwa.

Chemical substances, including heavy metals, introduced into aquatic ecosystem can disturb the homeostasis of a habitat. The aim of this study was to assess the effects of cadmium compounds on common carp, *C.batrachus* L. and to follow the toxicodynamics of cadmium elimination from intoxicated fish once they were transferred to a clean ambience

Fish is a dependable source of animal protein in developing countries like India. Large-scale mortality occurs among the fresh water fishes often due to environmental stress followed by pathogenic attacks and parasitic afflictions. Sreedevi P et al. (1992)[186] told nickel concentration, increased significantly in the gill, kidney, liver, brain and white muscle of the freshwater fish, Cyprinus carpio, and in the ctenidium, hepatopancreas, mantle, adductor muscle and foot of the freshwater mussel, Lamellidens marginalis, at 1, 2, 3 and 4 days on exposure to lethal and at 1, 5, 10 and 15 days on exposure to sublethal concentrations of nickel. Zaman Najmuz et al. (1999)[202]. Study LC50 dose for 24 hours and 48 hours duration of cythion on *Clarias batrachus* was determined by static bio-assay method. The quantity of cythion required was 17.16 mg/l and 15.6 mg/l for the two duration. The safe concentration was 5.63-mg/l. Sarkar SK (1999)[163] study on The fish *Cyprinus carpio*, when exposed to mixture of copper sulphate

and cadmium sulphate at different ratios (1:1, 2:2, 3:3, 4:4, 5:5) exhibited less oxygen consumption than individual metals. At 0.320 and 0.317 mg/l of copper sulphate and cadmium sulphate, the oxygen consumption of fish decreased by 9 and 12% of the control respectively. Sarvana Bhavan P et al (1999)[164]. study on Juveniles of *Macrobrachium malcolmsonii* were exposed to a median lethal concentration (96 hr LC50 : 12,589 mg/L) of dichlorvos for a duration of 96 hr. Sampling was performed on the gills, hepatopancreas and muscle of the prawns at 24, 48, 72 and 96 hr. Decline in concentrations of total glycogen, protein and lipid were noted in the test prawns in comparison to controls. The activity of acetylcholinesterase and alkaline phosphatase were found to be lower in the test prawns in comparison to controls. Selvarajan V.R. et al (1992) work on The biochemical markers such as DNA, RNA and protein have been analysed to study their quinalphos toxicity in different tissues such as brain, liver, muscle and gill of fish Oreochromis mossambicus. The fish were exposed to LC50 concentration of quinalphos and analysed DNA, RNA and protein at the end of 24, 48, 72 and 96h. Results revealed heterogenous trend of DNA, RNA and protein. The significant alterations of the biochemical constituents in various tissues indicated the toxicity of the pesticide. US.Sinha et al (1999)[193] work on LC50 dose for 24 hours and 48 hours duration of cythion on *Clarias batrachus* was determined by static bio-assay method. The quantity of cythion required was 17.16 mg/l and 15.6 mg/l for the two duration. The safe concentration was 5.63 mg/l.

1.1 Background :

Due to rapid industrialization and urbanization, large amounts of heavy metals and their compounds are continuously releasing the riverine system of India in general and of the industrial areas province in particular. These points towards desperate need for assessing the problem and to develop methods for alleviating the ill- effects of pollutants like lead and nickel because polluted water can cause paralysis, meningitis, cancer,

sterility, schistosomiasis, poliomyelitis and filariasis in animals. Therefore, the present study was planned to assess the metals, viz. cadmium, mercury and nickel toxicities in fish and water of river Betwa and Pahuj.

Chemical substances, including heavy metals, introduced into aquatic ecosystem can disturb the homeostasis of a habitat. The aim of this study was to assess the effects of cadmium compounds on common carp, *Cyprinus carpio* L. and to follow the toxicodynamics of cadmium elimination from intoxicated fish once they were transferred to a clean ambienc. Banerjee V et al. (1998)[15]. The changes in blood parameters due to lethal and sublethal exposures of mercury and zinc on *Heteropneustes fossils* were reported. There was no change in erythrocyte shape, size and surface areas of erythrocyte and its nucleus. Erythropenia associated with hypochromasia, increase in ESR, leucocytosis increase in large lymphocytes, thrombocytosis and hypercoagulability of blood were observed. Sesha Srinivas V et al. (1999)[172], attempt is made to assess the effect of 96h LC 50 concentration of hexavalent chromium (39.40 mg/l) on the oxygen consumption of the widely cultured freshwater fish, *Labeo rohita*. Alterations are observed in the metabolic rate of the fish exposed to chromium and the metal is found to be a potential respiratory inhibitor.

1.2 Metals in the Environment:

Metals play an important part in modern societies and have historically linked with industrial development and improved living standards. Society can draw on metal resources from Earth's crust as well as from metal discarded after use in the economy. Industrial society values metals for their many useful properties. Their strength makes them the preferred material to provide structure, as girders for buildings, rails for trains, chassis for automobiles, and containers for liquids. Metals are also uniquely suited to conduct heat (heat exchangers) and electricity (wires), functions that are indispensable to industrial economies. Finally, metals

and their compounds are used for their chemical properties as catalysts for chemical reactions, additives to glass, electrodes in batteries, and many other applications. The basic and unique properties of metals, including the ability to work them into complex shapes (i.e., ductility), insure that long term demand for metals will certainly grow

In the heavy metals and their compound that are harming the aquatic life, the important ones are mercury, lead, chromium, cadmium, silver, nickel, zinc, copper and iron. Excess heavy metals are often introduced in to aquatic ecosystem as by product of industrial and acid mine drainage residues. Blaise and Costan (1987)[20] have analyzed and assessed the toxicity of 300 effluent samples collected from 160 different industrial sites including 6 industrial sectors: mining, textile, food, pulp and paper, chemical and refinery. Environmental pollution by heavy metals was instantly recognized with Takeuchi et al. (1962)[189], where several thousands of people suffered mercury poisoning by consuming the fish caught in Mimamata Bay, which was recipient of mercury released from a vinyl chloride plant between 1953-1960. The available literature deals with the toxicity of various metals and other pollutant to some fresh water fish. Since the massive outbreak of mercury poisoning in Minimata Bay of Japan, mercury has been recognized and reported by Kastuki et al (1957)[86], Takeuchi et al (1962)[189], as on of that most hazardous environment pollutant. AB Gupta, et al. (1997)[1] determine the LC 50 values at 24, 48, 72 and 96 hr for the Indian catfish Heteropneustes fossilis ranged between 29. 50 and 17. 00 mg/l for Metasystox; 146. 00 and 124. 50, mg/l for Glyphosate; 1. 110 and 0. 740 mg/l for Karathane; and 0. 034 and 0. 026 mg/l for Decis

1.2.1 **Morphology of *Labeo rohita*** :

Labeo is a large essential tropical genus of Carps distributed in tropical Africa and East India. About two dozens of species are known from India, the most common being *Labeo rohita* (rohu) and *Labeo* calbasu which occur almost throughout India. Pakistan and Bangla Desh. Rohu is commonly found in river, lakes and estuaries. It prefers cleans water and respires by means of gills. It is chiefly hervivorous and a bottom feeder eating algae and aquatic plants. Body is spindle shaped. Colour is grayish or blackish on back and silvery white or pale on the two sides and belly. A full grown individual measures 1 meter in length and **20 to 25 kg in weight**. The weight body is divisible in to head, trunk and tail.

1.2.2 **Morphology of *Clarias batrachus*** :

The **walking catfish** (*Clarias batrachus*), also known as the **magur** or **pla duk dam**, is a species of airbreathing catfish with the ability to "walk" out of the water and across land. Its "walk" is more like a sort of wriggling motion with snakelike movements, as well as using its pectoral fins as "legs". This fish normally lives in slow-moving and often stagnant waters in ponds, swamps, streams and rivers (Mekong and Chao Phraya basins), flooded rice paddies or temporary pools which may dry up. When this happens, its "walking" skill comes in handy for moving to other sources of water.

Walking catfishes are around 25 cm (a foot or so) in length and has an elongated body shape. This catfish has long-based dorsal and anal fins as well as several pairs of sensory barbells. The skin is scaleless but covered with mucus, which protects the fish when it is out of water

The walking catfish is a native of Southeastern Asia including eastern India, Sri Lanka, Bangladesh, Burma, Indonesia, Singapore, and Borneo. It was probably introduced into the Philippines. The catfish is a tropical animal and prefers a water temperature in the range of 10 - 28°C.

In the United States it is a no indigenous invasive species, which is noestablished in Florida and reported from California, Connecticut, Georgia, Massachusetts, and Nevada.

Chapter - 1

INTRODUCTION

Chapter – 2

LITERATURE REVIEW

Literature Review

It is now well realized that environmental problems have increased exponentially in recent decades mainly because of rapid growth in human population and increased demand for several household materials. While on one hand technological development has improved the quality of life, on the other hand it has created a number of health hazards. The toxic chemicals discharged into air, water and soil get into food chain from the environment. By entering into the biological system they disturb the biochemical processes leading to health abnormalities, in some cases to fetal consequences by Pratima Gupta, 1998. In 1975 U. S. Environmental Protection Agency (U S E P A), Occupational Safety and Health Administration (O S H A), Consumer Product Safety Commission (C P S C) listed 24 extremely hazardous substances. These include heavy metals also. One such important heavy metal is cadmium (Cd). Cadmium is a well-known cumulative poison in animals that belongs to group II-b of the periodic table. Cadmium enters surface water with the discharge of industrial wastes or by leaching of soil, to which sewage sludge is added. It is biologically very reactive and therefore gives rise to both acute and chronic poisoning. Nariagu (1983)[128] emphasized elaborately on effects of cadmium on aquatic organisms. Many reports are available on the effect of Cd on fish blood. Blood is a good bio indicator or a diagnostic tool to study the problem in organ function. The measurement of biochemical changes in blood of fish under exposure to any toxicant may be used to predict effects upon chronic exposure. Present work was a comparative study with a siluroid air-breathing catfish *Clarias batrachus*. Effects were studied on some serum biochemical parameters. Ramalingam V et al. (2000)[146] study on The effect of lead acetate at a sublethal concentration of 10 mg/L, for 30 days, was studied on haematological parameters and some biochemical changes in the liver of a fresh water fish *Cirrhina mrigala*. Lead acetate produced significant haematological and

biochemical abnormalities. Avasan Maruthi Y et al (1999)[14]. Study on the Variation in the rate of oxygen uptake in the fresh water fish *Channa punctatus against different concentrations of sugar mill effluents and at* three different flow-rates, using flow-through system are discussed. The fish were exposed to 1 to 5 percent of sugar mill effluents at the 0.3048, 0.6096 and 0.9144 m/sec flow-rates and rate of oxygen consumption was studied in comparison with that of control. During the period of exposure, the rate of oxygen consumption decreased gradually with the increase of effluent concentration, flow-rate and the duration of exposure. Kumaraswamy P et al. (1999)[103] Study deals with the alteration in the rate of oxygen consumption and filtration rate of an estuarine clam *Meretrix casta*, as a result of 96 hours of exposure to sublethal concentration (1 ppm) of cadmium in different salinities. The rate of oxygen consumption increased with the function of decreasing salinity. However the filtration rate decreased exponentially with the decreasing salinity. Margarat .A et al *(1999) study on the influence of penicillamine against mercury intoxicated* has been determined in the brain and muscle tissues of mice, *Mus musculus* through enzymological parameter. A significant inhibition in AchE activity was observed in both the tissues of mice when exposed to mercury. Administration of penicillamine restored the mercury inhibited enzyme activity in both tissues.

2.1 General:

In the pisiciculture water quality gives healthy environment for the fishes. The fish are highly sensitive to toxic chemicals. The toxicity of industrial effluent, especially of water-based industries has been area of great interest among the biologists. The industries have been discharging metallic pollutants in their effluents, which inum has been causing serious *diseases and even death of the aquatic animals. This gives attention of* the scientist, for the work out the nature and toxicity of the metals to the

aquatic organism. Duodoroff (1957)[49] and Sprague (1969, 1970&1971)[182183,184] collected the method data interpretation for marine and fresh water fish. Jagadeesan G, Vijayalakshmi S (1999)[51] study on Alterations in the behavior pattern induced by different concentrations of mercury is reflected in *Labeo rohita* fingerlings to acclimatize themselves to the toxic environment. At sub-lethal concentration, absence of locomotors activity, increased opercular movements and stationary action, following four days treatment (96 hours) suggests their ability to withstand the toxic environment. Despande et al. (1999)[43]. study on the enzyme responsible for conversion of cyanide to thiocyate, rhodanese was isolated from *B. cereus var mycoides*. The enzyme was partially purified and studied for typical enzymological aspects.

2.2 Pesticides and Insecticides:

Pesticides are substances used to prevent, destroy, repel or mitigate any pest ranging from insects, animals and weeds to microorganisms such as fungi, molds, bacteria and viruses. EPA licenses or registers pesticides for use in strict accordance with label directions, based on review of scientific studies on the pesticide to determine that it will not pose unreasonable risks to human health or the environment. EPA is reviewing older pesticides to ensure that they meet current safety standards and is taking action to reduce risks where needed. For pesticides used on food, EPA sets limits on how much of a pesticide residue may remain in or on foods. EPA also sets standards to protect workers who may be exposed to pesticides on the job. EPA works to promote a safer means of pest control through research, public education, and public-private partnerships. The Environmental Protection Agency is responsible for a number of activities that contribute to food security within the United States, in areas such as food safety, water quality, and pesticide regulation. The Agency is responsible both for the registration of new pesticides and the re-registration of older pesticides to ensure that they meet current scientific

standards. EPA sets "tolerances," which are the maximum amount of pesticides that may legally remain in or on food and animal feed, and works with the Food and Drug Administration and the United States Department of Agriculture to ensure that these limits are not exceeded

Many Scientists have described about the toxicity of pollutants in different species of fish parathion to fresh water fish, *Labeo rohita* and oxygen uptake rate of exposed fish. Their studies have revealed the 96h LC50 values of pesticides for the fish large fish gradual reduction in oxygen. In smaller fish showed increased opercular beats.

Chatterjee and Konar (1984)[30] had give the effect of diazinon pesticide ecosystem. Gluth and Hanke (1985)[64] has show the rise in serum glucose and cholesterol with the decline in plasma protein and cholesterol content in *Cyprinus carpio* by pollutant such as aldrin, toluene, methanol etc. Sastry and Siddiqui in 1985 have been shown the decreased rate of absorption of two sugars and amino acid in freshwater fish, *Chnna punctatus*. The toxic effects of Malathion and phosphamindon on the fish, *Channa striatus* have been evaluated by means of graphical method and probit analysis by Saxena and Phosphamides for 96 hours exposure were 0.35 and 10.47 mg/lit respectively.

Tucker and Leitzke (1984)[192] studied on the toxicology of insecticide for vertebrate wiled life and fish, he notice that produce changes in toxic values in various test systems, observed the factors causing differences between species and finally the factors were related to the differences in effects between chemicals under normal environment condition in the field. Chauduri, Sadhu and Mukhopadhyaya (1984)[33] have been studied.

The toxicity of two organophosphorus insecticides Malathion and phosphamidon to the fish *Channa striatus*. Virk S et al. (1999)[197] introduced The biochemical components of the liver of *Cyprinus carpio* L. were significantly reduced as compared to control following 60 days of exposure to safe and sublethal concentration of nickel (7.50, 15.00 mgl-1)

and chromium (10.00, 20.00 mgl-1) during various reproductive phases. The sublethal concentration of both the metals caused more deterioration of liver than the safe concentration

2.3 Fertilizer & other chemicals:

The toxicity tests on channel cat fish, I ctalurus puncatatus (Refinesque) fingerling were conducted with ammonium chloride and ammonium sulphate at four different pH levels by Sheeshan (1985)[175]. Ammonium chloride solution were found to be more toxic than ammonium sulphate solution and when compared in terms of sulphate solution and when compared in terms of equivalent chloride concentrations to ammonium chloride solution at a pH of 6.0, fingerling appeared to be more sensitive to potassium chloride and about equal in sensitivity to a physiologically balanced cation/ Chloride mixture.

Metals have toxic effects on aquatic plants and animals and can bioaccumulate in aquatic species, such as mussels, which can then have a dangerous impact all the way through the food chain. Trace metals, such as arsenic, copper, cyanide, mercury, nickel, and lead come from and even air emissions from far away factories. These metals are toxic to aquatic life and accumulate in the sediments of streams, lakes, and estuaries as well as in fish tissue. These metals may come from pesticides, industrial waste discharges, solid waste landfill leachate, agricultural waste, or corroding metal pipes and storage tanks.

2.4 Industrial pollutants:

Chatterjee and Bhatacharya (1984)[31] observed the effect of industrial pollutants to the climbing perch, *Anabas testudineus* (Bloch). It was demonstrated that in teleost, the pollutants influence thyroxine relase mediated via the peroxidase system of iodination.

There was a direct relationship between the concentration of industrial effluent and the chloride, ammoniacal nitrogen and carbon dioxide content of water, but no correlation with pH and dissolved oxygen. The effect of various concentrations (5, 10, 15 and 20 ppm) of electroplating waste on liver, gills and kidney glycogen and on serum glucose and lactate was studied in nine fish species. Glycogenolysis was higher in major carps than in catfish or *Clarious batracus*. The lowest level of glycogen was recorded in gills, followed by liver, kidney. There was an inverse relationship between the concentration of waste and the decrease in liver and gills glycogen. There was a direct relationship between the concentration of waste and levels of serum glucose and lactate, which were higher in major carps than snake headed or cat fish.

2.5 Metals:

Many scientists have undertaken the studies on the effect of metals in different varieties of fish. Heavy metals such as mercury, copper, cadmium, nickel etc are highly detrimental to public heath. These metals may be more insidious and serious pollutants than the organic pollutants.

The effect and distribution of heavy metals are studied at all levels of - quatic ecosystems contaminated with them. Komarovski and Polishtuk in 1981. detected larger metal loads in the tissues of predatory fish species. According to Tinisli (1982), there are 2 ways for penetrating into the organism. The metal concentration in striated muscles of 268 fish specimens were determined for As, Cd, Cu, Pb, Mn, Hg and Zn by Blevins and Pancarbo (1986)[21]. Toxic metals like As, Cd, Hg and Pb and polychlorinated biphenyl have been reported in free living fresh water edible fish by Klein, Smidt and Kotter (1986)[95].

2.5.1 Mercury and its compounds:

The effect of mercuric chloride on the blood and kidney of toadfish, Halo*batrachus didactylus* has shown some hematological and histpathological changes in the fish. Sarasquate et al. (1982)[162] and Sastry and Rao (1984)[166] were shown the effect of mercury chloride on some biochemical and physiological parameters of *Channa punctatus*. Kaviraj (1983)[87] has shown the effect of mercury on the feeding rate of the fish. Klaverkamp et al. (1983)[94] have studied the selenite toxicity and mercury selenium interaction in juvenile fish. Verma et al. (1985)[196] observed an interesting phenomenon on the effect of environmental variables such as temperature, dissolved oxygen and pH on the toxicity o mercuric chloride to the fish, Notopterus notopterus. Acute exposure to 181μg/l of mercuric chloride (96hLC50) lead to erythrocytic anomalies including vacuolation, nuclear deterioration, microcystosis and callapsed cytoplasmic membranes. There was significant thrombocytosis and neutopenia together with a slight reduction in lymphosite count. Mercury has wide distribution in the earth's crust and aquatic environment says by Ruvio, (1972)[158] and Zingde (1989). A number of studies have shown the distribution and accumulation of mercury in water, sediments, zooplanktons and fish by Zingde (1989), Kureishy et al. (1979)[105], Kureishy et al. (1983)[106]. Sanzgiry et al. (1988)[159] and Krishnakumar & pillai (1990)[97]. Mercury in various concentration of chloride was used to study the ecophysiology and mercury accumulation in rainbow trout, Salmo gairdneri by Walczak et al. (1986)[198]. Narain Ram and Sathyanesan (1986)[127]. Shows the effect of two mercury compounds on the protein, RNA and DNA contecnts in the brain, liver and ovary of the fish *Channa punctatus*.

Clarias batrachus, an important air-breathing fish, inhabits muddy, marshy, and derelict waters Günther, 1880. The air-breathing organ (ABO) (an accessory respiratory organ; ARO) in this fish is modified gill

experiments, mortalities occurred more during the initial hours than at the end hours of the experimental period. Aditya Ajit Kumar, Chattopadhyay Sajib, Mitra Shyama, (2002)[3]. adult pre-spawning fish *Labeo rohita* were sublethally (1/5th 96h LC50) exposed to mercuric chloride and metacid-50 (methyl parathion). Accumulation of mercury and methyl parathion was studied and it was found that pre-spawning ovary appears as a potent organ for deposition of both the pollutants. RNA / DNA ratio of the control and treated fish were studied. It swas found that the significant decrease in RNA / DNA ratio occurs after 9 and 30 days of exposure for mercury and 30 days for methyl parathion

2.5.2 Cadmium and its compounds

Heavy metals and their salts constitute a very important group of environmental pollutants since they are potent metabolic inhibitors. The inherent toxicity of a metal depends upon its capacity to disturb the dynamic life processes in biological system by combining with cell organelles, macromolecules and metabolites. Cadmium is considered as non-essential element. This study was performed to begin an assessment of effect of the heavy metal on biochemistry of blood serum because blood is a good patho –physiological indicator. Test animals used for the study were *Clarias batrachus* and *Labeo rohita*. Both the fishes responded differently to the same toxicant and for same duration of time. In *Clarias* there was a decrease in glucose, cholesterol, total protein, urea and creatinine value. While in Lebeo *rohita only* glucose and sodium showed a decrease but all the other parameters showed elevation in values. After studying the result of present work it is clear that Cadmium very much affects the energy metabolism, which in long term cause the death of the individual organism and affects the whole community. The studies on the accumulation of cadmium in human kidney cortex has been made by Pandya et al. (1985)[136] and the relationship of cadmium accumulation to

cardiovascular disease were reported by Murungi and Robinson (1985)[123].Shukla Vineeta, Rathi Pratima, Sastry KV (2002)[176]. Studies on the impact of metal cadmium on the nutritive value of *Channa punctatus* on exposure to a sublethal concentration (1.12 mg/l) of cadmium (Cd2*) for 15 and 60 days has been studied. Among the various parameters selected, the level of moisture in liver and muscle was increased, while decrease was noted in the level of ash, total proteins and inorganic constituents life iron, calcium, inorganic phosphate, sodium and potassium in both liver and muscle in the two types of exposure. The total lipid level of liver increased, while muscle lipid level was decreased.

2.5.3 Nickel and its compounds:

Some heavy metals are essential to living organisms but their excess can disturb the homeostasis of an animal. The aim of the study was to assess effects of nickel compounds on carp, *Labeo rohita* & *Clarias batrachus* L., and to follow the toxicodynamics of this metal elimination from intoxicated fish once they were transferred to a clean ambience.

Abrahan and Radha Krishnan (2002) studied on *Paratelphusa hydrodromus* (Herbst.) was exposed to 50 ppm nickel chloride solution for a period of 10 days under laboratory conditions. The gills of the treated and non-treated animals were sectioned, strained and examined under a compound microscope and noticed significant changes such as enlargement of gill lamellae, lifting up and rupture of epithelial cells, enlargement of mid rachis, hyperplasia and hypertrophy, appearance of pyknotic nuclei and a general necrosis in the treated gills. Results suggest that a comparatively low concentration of nickel (50 ppm) is enough to elicit pathological changes in *Paratelphusa hydrodromus*

Sarala Nair et al. (2000)[161] the effects of sublethal concentrations of mercury on the haematology of *Oreochromis mossambicus* were monitored. Very low concentrations of mercury induced significant

changes in the hierogram of the fish. TEC, Hb & MCHC recorded higher values in heavy metal exposed fishes. The major toxic effects are increased erythropoiesis and microcytosis.

It has been well demonstrated that a number of trace metals are essential for various biological functions, often at very low concentrations. They are critical in many of the enzymatic and metabolic reactions occurring within an organism. Many metals are components of prosthetic groups in proteins (e.g., cadmium, nickel) or cofactors in enzymes (e.g., manganese, molybdenum, selenium) Depledge and Rainbow 1990. The essential nature of an element is established when: (1) it is present in living matter; (2) it is able to interact with living systems; and (3) a deficiency results in a reduction of a biological function, preventable or reversible by physiological amounts of the element Mertz 1974. Studies on the nickel toxicity have been studied by Chaudhery and Nath (1985)[32] on *Colisa fasciatus*, fresh water teleost. These studies revealed the fact that the blood glucose level increased by the administration of nickel sulphate at the sublethal dose of 64 ppm for 3 to 96 hrs. There was a steady increase upto the maximum of 85. 08 % at 96 hrs, blood glucose level has been adjudged to be a reliable indicator of nickel toxicity to fish. Ney and Hassel (1983)[90] investigated interspecific variations in nickel, lead, cadmium and zinc concentration among six species of fish form a highway- contaminated stream, as function of differences in habitat and morphology. Ajmal et al. (1985)[5] from Delhi to Allahabad at 5 sampling stations using Eichhornia crassipes as plant and Heteropneustes fossilis as animal.

Nickel has been shown to be essential for a wide variety of animal species including chickens, rats, pigs, cows, sheep, and goats (for a review on terrestrial organisms see Phipps et al. 2002. The effects of nickel deficiency include delayed gestation period, fewer offspring, anaemia, skin eruptions, reduced hemoglobin and hematocrit values, and reduced activity of several enzymes (WHO 1991). Although a deficiency disease

for nickel in humans has not been identified, probably because the nickel supply in diets exceeds its requirement, there is substantial circumstantial evidence for the essential status of nickel Anke et al. 1995; Nielsen 1996. The essentiality of nickel has also been demonstrated for many bacterial and plant species, where eight nickel-containing enzymes present in one or more of these species have been identified e.g., Van Baalen and O'Donnell 1978; Gordon et al. 1978; Eskew et al. 1983; Pederson et al. 1986; Price and Morel 1991; Ragsdale 1998; Watt and Ludden 1999. However, the information on optimal and deficient nickel concentrations is limited. The essentiality of nickel to aquatic animals (invertebrate as well as vertebrate species) remains unknown, as a nickel-containing metalloenzyme has yet to be recovered from animal tissues Goyer and Clarkson 200.

The aim of the study described in this paper was to assess effects of nickel compounds on common carp, *Cyprinus carpio* L. exposed to nickel via an intraperitoneal injection, and to follow the toxicodynamics of nickel elimination from intoxicated fish once they were transferred to a clean ambience. Specifically, dynamics of absorption, transfer, and—especially—elimination of the toxic substance after a single intraperitoneal injection were followed and changes in the fish body during those processes were observed. Anand Kumar V (1985)[7] says a profound increase in Mean Corpuscular Volume (MCV) was observed in endosulphon exposed fish when compared to cypermethrin intoxicated ones. A decrease in MCHC was recorded in endosulphon exposed fish, while the values of same parameter were inconsistent in the case of cypermethrin treated fishes. Jyothi B. et al. (1999)[84] study on Freshwater edible fish, *Clarias batrachus* (Linn.) was exposed to sublethal concentration of carbaryl, a carbamate pesticide. Several histological disorders occured in gonads. Reduced gonodosomatic index of testis and ovary was observed. Besides vacuolation and necrosis other histopathological changes noticed were arrested ovarian recrudescence,

cessation of spermatogenesis, interfollicular oedema in ovary and thickened basement membrane in testis. Meenakshi V et al. (1998)[117] study on the sublethal effects of mercuric chloride on the liver glycogen, muscle glycogen, blood glucose and blood lactic acid were estimated in the freshwater fish, *Cyprinus carpio* fingerlings. Fingerlings were exposed to the sublethal concentration of mercuric chloride (3.75 C 10-4 mg/l) for a period of 30 days. Fluctuation observed in the metabolites of glycogen indicates the possible arrival of anaerobic condition of the exposed fingerlings.

Chapter – 3

EXPERIMENTAL PROGRAM

Experimental Program:

3.1 Material and methods:

In the present investigations, toxicity of heavy metals cadmium, mercury and nickel to fresh water *Clarius batracus* and mercury or nickel or Cd to has been studied. The procedure for the analysis of water, determination of median lethal conservation and determination of accumulation of heavy metals in fish tissues were followed from APHA1985)[8] and EIFAC (1983)[50].

3.2 Material:

3.2.1 Chemicals: the chemical used for the treatment given to the fish is:

a) Cadmium chloride
b) Mercuric chloride
c) Nickel sulphate

The chemicals were of analytical grade purity as packed by E. Merck. The chemicals used for the estimation of metal accumulation in fish were also of analytical grade purity from E. Merck, B.D.G.H., and S. Merck. These chemicals are: -

1. 95% ethyl alcohol
2. Anhydrous Potassium Suphate (Na2SO4)
3. Bromine Water
4. Chloroform (CHCl3)
5. Concentrated Acetic Acid (CH3COOH)
6. Concentrated Ammonium Hydroxide (NH4OH)
7. Concentrated Hydrochloric Acid (HCl)
8. Concentrated Nitric Acid (HNO3)
9. Concentrated Sulphuric Acid (H2SO4)
10. Cupferron.
11. Cycloheptanedionedioxime (Heptoxime)
12. Disodium Hydrogen Phoosphate (Na2HPO4.12H2O)
13. Dithizone solution
14. Hydroxylamine hydrochloride (NH2OH.HCl)

15. Mercuric Chloride ($HgCl_2$)
16. Methyl orange
17. Nickel Sulpahte ($NiSO_4.6H_2O$)
18. Potassium Bromide (KBr)
19. Potassium Cyanide (KCN)
20. Potassium Permanganate ($KMnO_4$)
21. Sodium Hydroxide (NaOH)
22. Sodium Permanganate ($K_2S_2O_8$)
23. Sodium persulphate ($K_2S_2O_8$)
24. Sodium Potassium tartarate ($NaKC_4H_4O_6.4H_2O$)
25. Sodium tartar ate ($Na_2C_4H_4O_6.2H_2O$)
26. Tartaric Acid ($H_2C_4H_4O_6$)
27. Thymol Sulfonephthalein Sodium Salts

3.2.2 Equipments:

1. Plastic pool: The plastic pool with 3-meter diameter and 50c.m. Depth was used for the acclimatization of the fish

2. Auauaria: Glass aquaria measuring 2' x 1'x 1' were used fro carrying out the experiments.

3. Aerator: Royal mark aerators were used for continuous aeration of he water in the aquarium during treatment given to the fish.

4. Thermostat: Thermostat and heater manufactured by dutta electronics, Jhansi were used for 25*c to 27*c in the aquarium. They were slandered before used.

5. Spectrophotometer: Spectronic 20 spectrophotometer (Baush and Lomb make) was used.

6. Separatory funnels: 125 ml, 250ml and 1000ml with TFE Stopcocks. 125 ml, squibb from with ground glass stoppers.

7. Microtome: Weston Rotary microtome was used for section cutting.

8. Wintrobe ESR Tube:

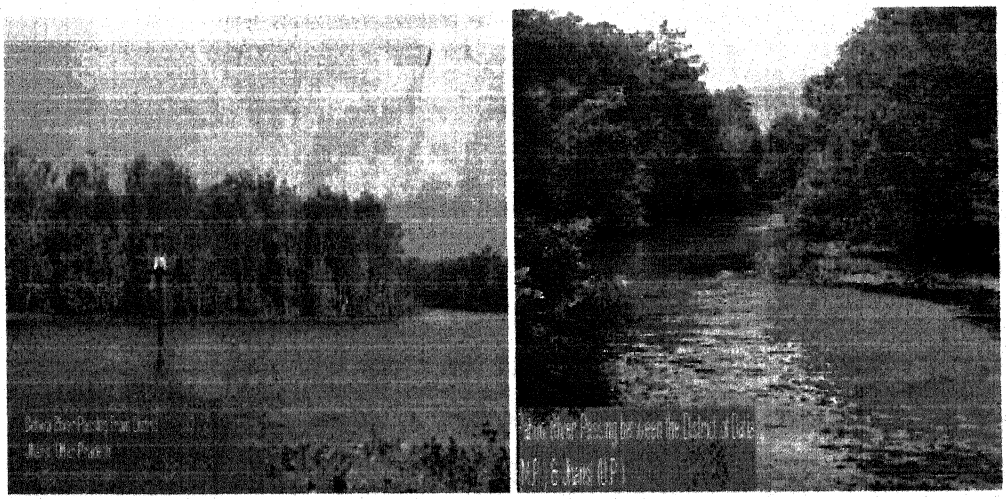
9. Neubaur Haemetocytometer:

10. Sahali's Haemoglobunometer.

3.3 Method:

3.3.1 Test fish and their collection:

The fish on which the effects of metallic pollutants have been studied are *Labeo rohita* and *clarius batracus*. *Labeo rohita* were collected from Stream of Betwa River near Jhansi. Healthy, living specimens of *C. batrachus* in the weight range of weight of 58 ± 2g and length of 18 ± 3 cm were collected from local market. The fish were handled very softly to avoid injury to them. Prior to experimentation the fish were allowed to acclimate to laboratory conditions for 2 wk. The fish were fed twice daily with commercial balanced fish feed in the form of pellets at the rate of 3% of body.



During acclimation they were fed with minced goat liver every day (d), for 3 hours (hrs). Water was renewed after every 24 h with routine cleaning of the aquaria, leaving no faecal matter, dead fish (if any) or unconsumed food. Prior to the commencement of the experiment, 96 hrs median lethal concentration (96 hrs LC50) of cadmium chloride E. Merck, India to *C. batrachus* was estimated following the Trimmed Spearman Karber method

HAMILTON et al., (1977)[70] and 24 hrs renewal bioassay system, and was 1gm found to be 70 ppm (95% confidence limit). For the analysis of sublethal toxicity, 24 hrs renewal bioassay systems were followed. Six groups of 10 fish each were exposed separately in six separate aquaria (marked as 10d, 20d, 40d, 60d, and two extras as ext-1 and ext-2) containing100. liters (L) each of 7-ppm (10% of 96 h LC50) cadmium chloride solution prepared in well water having dissolved oxygen 5. 8 ppm, pH 7.4, water hardness 30.0 mg/L ANONYM. 1992 and a water temperature of 27 ± 2 0C. Parallel groups of 10 fish each were kept in separate aquaria containing 100 L of well water (without the addition of cadmium chloride) as controls. Feeding was allowed in the experimental as well as control groups every day for a period of 3 hrs before renewal of the medium throughout the period of the experiment. Random checking of goat liver for the presence of cadmium on 10 different days during the experiment did not reveal any detectable amount. After the expiry of 10th, 20th, 40th and 60th days of exposure, 3 fish each from the respectively marked experimental, as well as control aquaria, were sacrificed. For estimating the cadmium content, first a pair of gills, kidneys and liver was excised from the experimental fish as well as control fish separately and the tissues were placed in separate Petri dishes to dry at 80 °C until reaching a constant weight. Five hundred mg each of the dried tissues were placed in separate digestion flasks and nitric-perchloric acid (4:1) mixture was added. The digestion flasks were gradually brought to and kept at 130 °C on a hotplate until all materials were dissolved and the digests were diluted with deionized water. All the dissection instruments and glassware were acid washed and rinsed with deionized water. Metal concentrations in samples were measured using a Perkin Elmer A Analyst 800 atomic absorption spectrophotometer and is given in ppm. Obtained data were subjected to standard statistical processing based on random sampling of three different samples of experimental, as well as control groups, of each tissue at each sampling period. One-way analysis of

variance followed by Duncan's multiple range test was performed BRUNING and KINTZ, (1968)[26] to determine whether the bioaccumulation of cadmium in the various tissues studied was influenced significantly by the exposure periods. Since there were no significant variations in the values of the respective control tissues collected at the various exposure periods, the average value of each of the control tissues was taken into account.

3.3.2 Acclimatization:

The collected fish were first treated with 0.1 $kmno_4$ solution for removal of any possible fungal infection. The fish were acclimatized in the ordinary tap water in the plastic pool for 10 days. Under laboratory condition. The similar tap water was used in the aquarium during fish treatment. The fish were fed once a day on standard fish food. These were starved for 24 hours before being used for bioassay test.

3.3.3 *Preparation of stock solution:*

100µg of mercuric chloride ($HgCl_2$) was dissolved in 1 liter of distilled water (1N = 100mg HgCl2). For cadmium, 1gm. of cadmium sulphate was dissolved in distilled water (1ml = 1mg $CdCl_2$). For nickel, 1g of nickel sulphat ($NiSo_4$) was dissolved in 1liter of distlled water (1ml = 1mg $NiSo_4$)

3.4 Toxicity Tests:

To determine the toxicity of cadmium chloride, mercuric chloride and nickel sulphate, Bioassay tests were conducted screening tests were arranged prior to bioassay test, to ascertain the concentration of these metallic pollutants to be used for final bioassay test

3.4.1 Bioassay Test:

For chronic toxicity and histopathological studies long-term bioassay test were conducted while for acute toxicity of the metal was tested by short-

term bioassay test. Dead fish were removed immediatately and the time and number of their mortality was recorded.

3.4.2 Screening test:

Seven widely spaced concentrations of each salt were tested to find out the toxicity range of the metal concentrations. Three fish used for this preliminary-screening test. Results of screening test were used to decide the range of the concentration of the metal salts to be used for toxicity test.

3.4.3 Cadmium Treatment:

Starved fish for 24 hours were placed to aquarium containing different concentration of cadmium chloride, ranging from 0 mg/lit to 5.0 mg/lit. Each aquaria containing 20 liter of water, ten fish in each aquarium. Those fish, which are found in cadmium chloride, free aquaria (0 mg/l) for control set. The size of the fish ranged from the weight of the fish "*Clarias batrachus*" is ranged 58 ± 2g gm. The fish were observed after intervals of 24 hrs, 72hrs, 96hrs, 10days, 20days and 30days for the following.

a) Percent mortality was recorded with time by using short-term toxicity tests.
b) The change in the behavior of the fish was also observed.
c) The growth of the fish in terms of length and weight was recorded and compared with the controlled fish.
d) The fish were dissected and gills, liver and kidney were preserved in 20% neutral fromaline for histopathological studies.
e) The organs gills, liver and kidney were taken out and preserved in concentration HNO3 for the estimation of metal accumulation in these organs APHA (1985)[12].

3.4.4 Mercury treatment:

The toxicity of mercury has been studied by repeating the above experiment using mercury as mercuric chloride. It has been used in different concentration ranging from 0 µg/l to 1000 µg/l. 20 liters of water used in each of the aquaria mercuric chloride for toxicity test. The size of the fish *Labeo rohita* ranged 65 mm in length and the weight ranged 6.284 gm the fish were observed after the intervals of 24 hrs. 48 hrs, 72 hrs, 96hrs, 10days, 20days and 30days. In mercury treatment the observation have been reported for 23rd and 25th days, as mortality was observed on these days. All the observation from a to e were made as in cadmium treatment.

3.4.5 Nickel treatment:

Nickel is found in plating, metal pickling and metal cleaning wastewater. The nickel from the plating wastewater can be chelated from as nickel sulfamate from sulfamate nickel plating, or nickel lactate from electroless nickel plating. Nickel cleaning and stripping solutions are often having nickel cyanide and nickel EDTA complex. Nickel that isn't complexed or chelated can be precipitated as nickel hydroxide by adjusting the pH to 10.5 or higher. Complexed nickel must be treated with sulfide or other strong reducing agents that can maintain a negative ORP of –700 mv or less. Even then the treatment may be difficult to reach acceptable limits. Precipitating the nickel as a carbonate is not effective due to the high solubility of nickel carbonate. The precipitation of nickel phosphate is effective for lightly complexed nickel using a two-step process described in the copper section. To precipitate the nickel as the metal using ORP, a strong reducing agent, such as ferrous ion or sulfide ion, must be used at a pH greater than 10.5. Raising the pH to 11.5 and allowing the nickel to plate out using the residual reducing agent, sodium hypophosphite, in the solution before continuing the treatment should pretreat Electroless nickel solutions. The plating out or dropping out of

electroless nickel solutions releases significant quantities of hydrogen so the treatment vessel must be exhausted to keep the hydrogen below the explosion limits.

Nickel can be removed with cation resins and chelated anion resins. Using a reducing agent and sand filter or other media column to precipitate the nickel metal on the surface of the media can attain low concentrations. If nickel concentrations below 1 mg/l are required, a three-step process of precipitation, oxidation and then final precipitation or adsorption will be required. The treatment equipment for removing nickel is similar to that used for copper adding forced exhaust to prevent hydrogen build up during the treatment of electroless nickel baths. The toxicity of nickel sulphate. Defferent concentration ranging from 0 mg/l to 12 mg/l were maintained in glass aquaria containing 20 liters of water and ten fish in each of them. The fresh water *Clarias batrachus* ranging in size is 18 cm. and in weight is 58 ± 2g gm were to different concentration of nickel sulphate. The fish were observed at the intervals of 24 hrs, 48 hrs, 72 hrs, 96 hrs, 10 days, 20 days and 30 days. All the observation from a to e were made as in cadmium and mercury treatment.

3.5 Physico Chemical Analysis of Water:

Physico-chemical analysis of water was done every 24 hrs to maintain the standards of the test. The mean values of physico chemical properties of water are given in the Table No.1

3.6 Mortality:

Those fishes, which show no activity and not respond to mechanical stimuli, were counted for mortality. The number of dead fish was recorded in each concentration with time and were removed immediately form the test aquarium.

3.7 Analysis of results of toxicity tests:

Data, which are collected from different sets of toxicity, were processed to determine the lethal threshold concentration and median lethal concentrations for 24 hours, 48 hours, 72 hours and 96 hours exposure durations.

3.7.1 Lethal threshold concentration:

That minimum concentration of the metallic pollutant used, that caused the firsts death of the test fish, was recorded as lethal threshold concentration.

3.7.2 Median Lethal concentration:

It is term as *LC50* value and the concentration of the pollutant, lethal to one half of the test population of fish EIFAC 1983)[50] it is determined the for the test durations, such as 24 hrs LC 50, 48 hrs LC 50 etc. The percentage mortality was plotted on ordinate on probit scale and concentration of the metallic pollutant on the abscissa on logarithimic scale. Finney (1971)[57] and Sprague (1973)[185] suggested of LC 50, Probit analysis.

3.7.3 Toxicity curve:

Using LC50 values of different metal salts for different exposure durations does it. Plotting exposure time on ordinate and corresponding LC50 Values on abscissa plots curves on logarithmic graph paper.

3.8 Histopathology:

Histopatholgical studies were undertaken to find out pathological changes in cytoarchitecture of different organs. The fish for this study were kept in varying concentrations and were sacrificed after different exposure times. The organs liver, kidney and gills were studied for histopathological changes. These organs were fixed in 20% neutral formaline and paraffin

section of 6-8lμ thickness were cut. For histopathological studies the sections were stained with Delafied's Hematoxylin and Eosin.

Organ tissues, liver, kidney, gill and collected in 10% neutral buffered formalin were processed for paraffin blocks (56-58 °C) and sectioning at 3-5 μm. Stained sections were examined under a Zeiss compound binocular microscope (Axiophot, Germany) fitted with a photomicrographic attachment. Kumar Ravindar (2000)[101] studied on the effect of chronic exposure to a test concentration (70 ppm) of ammonia has been observed in kidney of teleost fish *Channa punctatus* (Bloch). In kidney, proximal tubules were highly affected. The nucleus in all the tubular cells was pycnotic and cytoplasm was granular. It was observed that changes at 28 days were more gradual in comparison to 14 days of ammonia intoxication.

3.8.1 Stains:

Delafield's hematoxylin and Eosin were used for routine double staining process for histological studies. They were prepared by the following method adopted from Johansent (1940)[81]

(a) Delafield's Hematoxylin: It was prepared by adding drop by drop a solution of 4g. hematoxylin in 25 ml of 95 % ethyl alcohol to 400 ml of saturated aqueous solution of ammonium aluminium sulphate. It was kept exposed to light and air for 10 days for ripening. 10 ml of c. p. Glycerine and 100 ml of methyl alcohol was added to it. It was allowed to stand for a period of 1 month exposed to air. The colour became sufficiently dark. It was mixed with equal amount of distilled water.

(b) It is a fluorance derivative with 2.18 % solubility in alcohol. It is a cytoplasmic stain for animal tissues. It was prepared by dissolving 1gm.of Eosin in 100 mll of 95 % ethyl alcohol. The deparaffinised section of tissues were passed through down grade series of alcohol

and finally brought to distilled water. They were treated further in the following manner.

a. Haematoxylin for 2 min.
b. Tap water for 2 min.
c. A dip in Acid water
d. Distilled water for 2 min.
e. 30 % alcohol for 5 min.
f. 50 % alcohol for 5 min.
g. 70 % alcohol for 5 min.
h. 90 % alcohol for 5 min.
i. Eosin solution for 1 min.
j. A dip in Acid alcohol
k. A dip in n90 % acohol
l. 100 % alcohol for 5 min. 1st change
m. 100 % alcohol for 5 min. 11nd change
n. Xylol for 3 min.
O Mounted in DPX.

3.9 Haemetology:

The fish *Labeo rohita* were netted from the Betwa River passing from Jhansi district in the first week of each month, from Sept. **2005** to Oct. **2005,** between **8-9** a.m. Blood was collected immediately after capture by severing the caudal peduncle. Heparin **(0.1** mg 1 ml-l of blood) was used as anticoagulant. The erythrocyte count was determined by an improved Neubauer haemocytometer with Yokoyama's solution as the diluting medium. Blood haemoglobin was estimated colorimetrically following Wong's method. Haematocrit was measured by Wintrobe Haematocrit. Leucocyte counts were made using a Neubauer haemocytometer, diluted in Yokoyama's solution. Clotting time was determined by taking blood direct from the fish in a capillary tube of **0.5** mm diameter.

The fish *Clarias batrachus* were collected and the similer procedure was adapted for haemetological studies were made. Disease-free fish, *Clarias batrachus* were bathed in 1% KMnO4 solution and acclimatised in big glass aquarium of Glass aquaria measuring 2' x 1'x 1' capacity for a period of 72 hrs. The healthy fish of both the sexes and uniform length and weight (18c.m. and 58.500 gm ±) were selected from the lot for the experimental purpose. Initially 24 hrs 96 hrs LC50 doses were determined for nickel heavy metal compounds by the method as described in standard methods by APHA, (1998)[13]. The fishes were divided into three groups.

Group- I: Consisting of 15 fish in aquarium. Water was changed, every day in the morning after removing the unused food. The controls as well as experimental fish were sacrificed on the day 10, 20 and 30. The blood was collected into vials containing heparin as anticoagulant by severing the caudal peduncle, after 10, 20 and 30 days of exposure. Haemoglobin (Hb%) was measured by Sahali's haemoglobinometer, RBC (TEC) and WBC (TLC) were counted by using Neubaur's haemocytometer.

Group- II: Consisting of 15 experimental fish exposed to sublethal dose of nickel sulphate (4mg/l) for 30 days.

Group-III: Consisting of 15 experimental fish exposed to sublethal dose. The fish were exposed to 4 mg/l of nickel sulphate, which are the 1/10th of their 96h LC50 concentrations. To avoid the effects of starvation, the fish were fed on the rice bran at the average feeding rate of 25 mg food / gm fish / day. WBC were counted by Neubaur's haemocytometer and Hayem's and Tuerk's solutions as diluting fluids, respectively and Packed cell volume (PCV) by Wintrobe's method (300 rpm for 1 hour). Differential leucocyte count (DLC) was carried out by preparing a thin blood smear and staining it with Leishman's stain. Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean cell volume (MCV) were calculated using standard formulae (Dacie and Lewis, 1982)[39].

3.10 Growth rate:

The fish growing at normal rate is presumed to be healthy while the condition of food and other things like temperature, quality of water are kept natural. The growth of the fish, C.*batrachus* and L. *rohita* maintained in aquarium with water without metal is measured in length and weight in 30 and 20 days and is registered as normal growth. These fish sp. were subjected to subleathal concentration of cadmium sulphate, nickel sulphate for 30 days and mercuric chloride treatment was given for 20 days. The growth of these fish in terms of weight and length was compared with that of control fish.

Chapter – 4

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

Macroscopic (behavioral) observation. On exposure to the sublethal concentration of cadmium chloride, the toxic stress on the fish was manifested in the form of restlessness and jerky and erratic swimming movements. The exposed fish also showed increased ventilatory movements of operculam and increased gulping activity. Instantaneous secretion of excessive mucus all over the body surface of the exposed fish was also noticed. Copious amounts of mucus were later released into the media at various stages of exposure in the form of streaks, along with rejected flakes of epithelial cells and other cell debris. Even though the exposed fish rejected the food provided, especially in the earlier stages (up to 5th day) of exposure, they started consuming the food (with hesitation) from the 6th day onwards and gradually resumed feeding activity to near normal situation by the 13th day. Throughout the duration of the experiment no significant behavioral or macroscopic changes were observed in the control groups. No death occurred either in the control or in the experimental groups during the whole period of the experiment.

Cadmium accumulation study. A summary of the analysis of variance showing the level of significance of accumulation of cadmium in the various tissues studied is documented in Table 2 along with the alterations in the pattern of bioaccumulation in Table 3 as revealed by Duncan's multiple range tests.

Liver. As with the case of gills, cadmium could not be traced in the liver of the control fish. In the case of the experimental groups, even though the quantity of accumulated cadmium was less in the case of liver when compared to gills, the pattern of accumulation showed a more or less continuous increasing trend until the 40th day of exposure in Table 3; Fig. 1. However, after 60 days the quantity of cadmium in the tissue decreased. The mean rate of accumulation was 0.77 ± 0.01 ppm (Fig. 1).

Kidneys. The rate of accumulation of cadmium in kidneys increased along with exposure time, with an exception only after 40 days (Table 3). The

mean rate of accumulation of cadmium in kidneys during the sublethal exposure was 0.81 ± 0.01 ppm, which is next to that of gills. Cadmium was not detected in the control fish. Traces of the metal were present in the case of the experimental tissue (Table 3). The rate of accumulation was less when compared to other tissues, such as gills, kidneys and liver (Table 3; Fig. 1). In the case of the experimental fish, the rate of accumulation decreased during the later periods.

4.1 Toxicity of metallic pollutants on Carps

Metallic pollutants cadmium chloride, mercuric chloride and nickel suphate were used to assess the toxicity of the metals to the fresh water fish, *Clarias batrachus* and for *Labro rohita*. The toxicity of metals was determined by using short term and long-term static bioassay test under different test conditions. In static bioassay test, the acute toxicity was ascertained by using different concentration of metallic pollutants. Ordinary water was used in the aquaria. The physico chemical characteristics of water used in the aquaria during bioassay tests are summarized in the pera 3.5, Table -1.The temperature of the water was maintained in the aquarium by using heater in conjunction with a thermostat in the winter and cooler in the summer.

The mortality of the fish was recorded with time and concentration of the metal, in which the death of the fish was noticed. LC50's were determined for different test duration, 24 hrs, 48 hrs, 72 hrs, and 96 hrs. By plotting results of bioassay test on log-profit graph, APHA (1985)[12], EIFAC (1983)[50], Finney (1971)[57] and Sprague (1973)[185]. The results were compared with the control fish, which were maintained in metal free normal water

4.1.1 Acute toxicity of Cadmium Chloride to *Clarias batrachus*

For the determination the acute toxicity of cadmium, different concentrations of cadmium chloride were used in the static bioassay test.

The range of cadmium chloride concentrations used was 1.0mg/l for control to 5.0mg/l. the result of the bioassay test are summarized in Table-4 and presented in fig.2. In fish, the toxicity of cadmium is also well known. Exposure of freshwater fish to cadmium leads to a disturbed calcium metabolism, resulting in hypocalcaemia and anomaly of the bone Therefore, cadmium disrupts calcium homeostasis in fish as well as humans. Although many factors might affect the results of tests of the toxicity of cadmium to aquatic organisms Sprague 1985, water quality criteria can quantitatively take into account only factors for which enough data are available to show that the factor similarly affects the results of tests with a variety of species. Hardness is often thought of as having a major effect on the toxicity of cadmium, although the observed effect may be due to one or more of a number of usually interrelated ions, such as hydroxide, carbonate, calcium, and magnesium. Hardness is used here as a surrogate for the ions, which affect the results of toxicity, tests on cadmium. The BLM, which quantifies the capacity of metals to bind to the gills of aquatic organisms, can be used to calculate the bioavailability portion of dissolved metals in the water column based on site-specific water quality parameters such as alkalinity, pH and dissolved organic carbon U.S. EPA 1999b. Future development of the BLM for cadmium will help better quantify the bioavailability fraction of cadmium. Yellamma K et al. (2000)[201] study on the cholinergic system of different brain areas showed variable sensitivity to cadmium chloride stress. In both acute and chronic dose studies the enzyme acetycholine esterase was inhibited significantly in all the areas. During acute dose studies maximum inhibition was noticed at 12th in all the brain areas, while in chronic dose studies peak inhibition was noticed on 7th day and recovery towards normalcy was noticed in all the areas after 11th day onwards

During the course of experiments no mortality were recorded in both the types of fishes exposed to sub lethal concentration of Cadmium chloride. Certain changes were observed in the coloration, feeding behavior and

activeness of the fishes. Both the types of fishes initially became more active but later their activity ceases. In both the types of fishes coloration fades a little, fluctuating responses were observed in feeding behavior. Table shows the biochemical indices recorded from exposing *Clarias batrachus* to 2.5 ppm Cadmium chloride for 96 hours, 15 days and 30 days. Differences were measured against the control values determined under controlled laboratory conditions. The value of glucose shows a gradual fall of 54%, cholesterol, total protein, creatinine, urea and potassium values show a regular increase while sodium levels show an initial increase of 11% but at the end of experiment it lowers to .7%

According to David CV, Shrivastava VK et al. (2000)[40] a single intraperitoneal injection of cadmium chloride and mercuric chloride were administered to male mice *Mus musculus* (P) and the hypothalamic gamma-saminobutyric acid (GABA) and glutamate levels were quantified on 31st and 61st day. The GABA levels were significantly increased in all the treated groups after 30 and 60 days of treatment. But insignificant elevation was noticed in Pb NO3 after 30 days of exposure Kumarasamy P, et al (2000) says that *Clibanarius infraspinatus* was exposed to the sublethal concentration of 1 ppm of cadmium at different salinities for 96 hrs. The rate of oxygen consumption of the crab and the biochemical constituents were analyzed. The rate of oxygen consumption increased with decrease in salinity. The amount of free sugar and protein increased when the animals were exposed to low salinities, whereas the amount of lipid decreased with decrease in salinities. The LC values for 24 hrs, 48 hrs, 72 hrs and 96 hrs., exposure duration and threshold concentration are included in the Table 5 and fig.4, 5, 6, 7. in the acute toxicity. The lethal threshold concentration of cadmium chloride was found to be 0.5-mg/l. mortality rates were found to increase with the increase of concentration of cadmium chloride. In the test container having 3.0-mg/l cadmium chlorides there was an abrupt increase in the mortality where 60 percent of the fish died in 48-hrs.100 percent mortality was observed in 4.0 mg/l in 120 hrs.

While it was 100 percent in 6.0 mg/l in 48 hrs. LC value for 24 hrs. was found to be 4.6 mg/l, while it was comparatively quit low 2.31 mg/l for 96 hrs. in 48 hrs and 72 hrs LC50 value was found 2.85 mg/l and 2.62 mg/l respectively. LC value is decrease with increasing test durations is clearly indicative of increase of toxicity of metal with the increase of test durations. Toxicity curve is plotted in Fig. 34 for LC value against test durations on log-log graph shows the relationship between LC50 value and exposure durations as the LC50 value decreased with the increase in time. The result of analysis of variance is given in the Table 6. and mortality of the fish in different concentration has been presented in Fig.19.

4.1.2 Acute toxicity of Mercuric Chloride to *Labeo rohita*.

It has been determined by using static bioassay test. The approximate range in which the fish survived for some definite period of exposure was ascertained by screening test and then different concentration of mercuric chloride were maintained in aquaria ranging from 0 µg/l to 1000 µg /l. the first mortality was observed in 96 hrs. in 50 µg /l mercuric chloride in which 80 percent fishes survived even at the end of 30 days exposure. All the fish were died in 100- µg /l concentration of mercuric chloride at the end of 30 days. 100 percent mortality was observed in 48 hrs in 1000 µg /l of mercuric chloride concentration. In 600 mg/l concentration the last fish survived only up to 9 days and all the fish died on 10^{th} day. In the exposure of 700 µg /l and 800- µg /l concentration of mercuric chloride all the fish died in 96 hrs. The observations are values in the Table-7 and have been expressed in Fig.9. The LC Value for different exposure period 24 hrs, 48 hrs, 72 hrs and 96 hrs have been determined by plotting the values of percent mortality on probit scale on ordinate and concentration on log scale on abscissa APHA (1985)[12]. Fig. 10.11.12.13. The relationship has been observed between LC 50 values and exposure duration showing decrease in LC50 values with the increase in exposure

durations Fig. 35 .hrs LC50 values was observed to be 680 µg/l, 48 hrs LC 50 value was recorded to be 533 µg /l, 72 hrs LC50 value was recorded to be 516 µg /l while 96 hrs LC50 value was registered to be 428 µg /l. LC50 values for differ rent exposure time and threshold concentration have been given in Table-8. The result of analysis of variance are given in the Table-9 and indicated that the exposure duration as well as concentration affect the mortality of the fish, *Labeo rohita* and both the highly significant at significances level 0.001. different concentration of mercuric chloride has been represented in Fig.20. Radhakrishnaiah K. et al.(1992)[143] Blood glucose level increased in *Labeo rohita* after 1,2 and 3 days of exposure to lethal (1.0 µg /l) and 1, 15 and 30 days of exposures to sublethal (0.2 µg /l) concentrations of copper, with a corresponding decrease in its liver glycogen content and increase in the activities of liver glycogen phosphorylase and glucose-6-phosphatase. Ramalingam V, Arunadevy R (1999)[144] introduced the effect of mercuric chloride at two different doses, 2 µg /kg bw (low dose) and 4 µg/kg bw (high dose), i.p. for 15 days was investigated in the testis of adult albino rats. Acid phosphatase, alkaline phosphatase and ATP-ases were estimated in the testis extract by standard spectrophotometric methods. Acid phosphatase increased ($P<0.001$) in the high dose treated animals without significant change in the low dose treated animals. Ramalingam V, et al (1999)[145] introduced the effect of mercuric chloride at two dose levels: 2 µg /kg body weight (low dose) and µg /kg body weight (high dose) i.p., for 15 days, was studied in the liver of mature male albino rats. Mercuric chloride caused a marked reduction in the body weight with alteration in liver weight. Glycogen content, acid phosphatase and alkaline phosphatase activities were markedly increased in high dose treated animals without any significant change in low dose treated animals. . Ramalingam V, et al. (2000)[146] study on the effect of lead acetate at a sublethal concentration of 10 µg /L, for 30 days, was studied on haematological parameters and some biochemical changes in the liver of a fresh water fish *Cirrhina*

mrigala. Lead acetate produced significant haematological and biochemical abnormalities

4.1.3 Acute toxicity of Nickel sulphate to Clarius *batrachus*

The acute toxicity of nickel sulphate was studied by short-term bioassay test. Different concentrations of nickel sulphate have been found to cause varying toxic effects to fish, *Clarias batrachus*. Different concentrations of nickel sulphate were use din the range form 0 mg/l to 12.0 mg/l. the first mortality in 96 hrs. Exposure duration was observed in 1.0 mg concentration. 30 percent fish in these concentrations died at the end of 30 days exposure. Almost similar results were observed in 2.0-mg/l concentrations. In 4.0 mg/l concentrations. In 4.0-mg/l concentrations, 70 % fish died at the end of 30 days 80% fish died at the end of 30 days when they are place in 6.0mg/l. When these fishes are Capt in 8.0-mg/l concentrations then 90 % of fishes died in 30days. In 10.0 mg/l concentration 100% of fish died in 10 days. Mortality was observed in 12.0-mg/l nickel sulphate in 96 hrs exposure duration. The result are summerized in the Table 10 and presented in Fig.14. The mortality and concentrations of nickel sulphate were used to calculate LC 50 values. The percent mortality was plotted on probit scale on ordinate and concentrations of nickel sulphate on log scale on abscissa.24 hrs, 72 hrs and 96 hrs LC50 values were determined Fig.15, 16, 17 and 18. The 96hrs LC50 values were calculated to be 6.6 mg/l, 72 hrs LC50 values was calculated to be 7.25 mg/l, in 48 LC50 value 8.8 mg/l and in 24 hrs LC50 value was registered as 11.36 mg/l. The toxicity curve is mentioned in Fig.36 the relationship was observed representing the increase in toxicity with the increase in exposure. LC50 values and threshold concentration are given in the Table.11. The results of analysis of variance ANOVA are given in the Table 12. It indicated that both concentration as well as exposure duration affect the mortality of the fish *Clarias batrachus* and both are highly significant at significance level 0.001.

Effect of exposure duration on mortality of the fish in different concentration of nickel sulphate has been represented in Fig.21 and Selvanayagam M et al. (1991)[170] study on the Scale carp fingerlings were exposed to the sublethal concentrations of nickel 2.5.5.0 and 10.0 mg/l and chromium 15, 30 and 60 mg/l for 30 days. The vertebral deformities were observed with the help of radiographs and changes in the vertebral mineral content were also noted

4.2 Effect of Toxicity of Metals on Growth:

4.2.1 Changes observed in the fish due to cadmium Chloride treatment:

Observation was made on the variations in growth in terms of length and weight of the fish *Clarias batrachus* subjected to different concentration of cadmium sulphate. The concentration of cadmium sulphate ranged from 0 mg/l to 2.5 mg/l and maximum exposure time of 30days was taken. The results of experiment appear in the Table.13.

The controlled fish registered a growth of 0.316 percent in weight and 11.111 percent in length. The growth amongst the fish subjected to metal treatment did not show same magnitude. The fish affected by 0.5 mg/l cadmium chloride concentration showed 3.49 percent growth in weight while 3.17 percent growth in weight and length to more 1.69 percent and 2.98 percent respectively. The growth in weight was recorded only 1.29 percent and in length the growth was negligible in 1.5-mg/l cadmium chloride. The trend remained unchanged even in 2.0 mg/l concentration where in the fish underwent a loss in weight of the order of 0.87 percent in 30days while the loss in weight was 4.45 percent on the 30th day in 2.5 mg/l concentration. The growth in length was not traceable in the fish subjected to 1.5 mg/l to 2.5-mg/l concentration of the metal. There was a steep fall recorded in the growth in terms of weight and length depicting the increase in the toxicity with the increase in concentration of the metal

4.2.2 Changes observed in the fish due to mercuric Chloride treatment:

The fresh water fish, *Clarias batrachus* has been subjected to different concentration of mercuric chloride to study the effect of its toxicity on the growth of the fish. In one test container the fish were maintained in controlled conditions with 0 µg/l concentration and in other container the fish encountered with different concentration of mercuric chloride ranging from 50 µg /l to 400 µg /l for 20 days. The controlled fish reregistered the increase in weight by 0.316 percent while the length increased by 11.111percent. A decline in the increase of weight was noticed in the fish subjected to 50- µg /l mercuric chloride, where only 5.862 percent increase in weight was recorded and increase in length was more than controlled, which was in 12.5 percent. With further increase of concentration of mercuric chloride in test containers with 100 µg /l and 200µg /l, an increase in weight by 0.093 percent and 9.185 percent was recorded, however after this; 300 mg/l concentration caused the loss of weight in 20 days exposure by 0.099 percent and 0.133 percent. Although the change in the length was positive, there was a gradual decrease in the percent growth of length in fish subjected to increasing concentration of mercuric chloride. 6.25 percent increase in length was observed in 100 µg /l concentration. 6.25 And 11.764 percent increase in length was noticed in the fish subjected to 300 µg /l and 400 µg /l concentration of mercuric chloride. However an abrupt increase of 12.5 percent length was observed in fish, kept in 200 µg /l of mercuric chloride for 20 days. These values have been represented in the Table 14

4.2.3 Changes observed in the fish due to nickel sulphate treatment

The effect of nickel sulphate on the growth of the fish *Clarias batrachus* has been determined by using static bioassay test for 30 days. The fish were kept in test containers with varying concentration of nickel sulphate ranging from 0 mg/l to 6.0 mg/l. The changes in weight and length were recorded at the end of the test and were compared with the gain in weight

and length of the controlled fish. The observations are summarized in Table 15.

The increase in weight and length by 0.231 percent and 10 percent were observed in the fish kept in 1.0 mg/l nickel sulphate. This increase in weight of the fish registered a decline, which was determined to be 0.093 percent in 2.0 mg/l concentration. Further increase in concentration of nickel sulphate cause the loss of weight of the fish by 0.140. Percent in 4.0-mg/l concentration in 30 days. The fish in 6.0-mg/l concentrations registered a loss of weight by 0.099 percent. An increase in the length was recorded to be 11.76 percent at 1.0 mg/l and 6.25 percent at 2.0-mg/l nickel sulphate, while the change was not traceable at further higher concentration.

4.3 Toxic effect of metals on the blood composition of fishes

4.3.1 Toxic effect of metals on the blood composition of *Clarias batrachus*:

Effect of Nickel sulphate and Cadmium chloride separately to study alterations in haematological parameters. The results are shown in Table: 27 and 28. Haematological parameters like total RBC, Hb gm%, and PCV were decreased where as, total WBC counts was increased under these stress of heavy metals under investigation. On the contrary, nickel sulphate caused uniform reduction in RBC count and Hb content following all the periods of exposure of the experimental fish (Table 27). In nickel chloride exposed fishes slight improvement in TEC and Hb% was seen after 30 days of treatment. The percent decrease in TEC after 20 days was 10.18% in nickel chloride exposed fishes, however after 30 days this decrease was just 6.11% (Table 27). Number of increase of Erythrocyte of *Clarias batrachus* after 10 days exposure to 4.0 mg/l nickel sulphate showing in P-35 and Number of decrease of Erythrocyte of *Clarias batrachus* after 30 days exposure to 4.0 mg/l nickel sulphate showing P-36 and37 in cadmium chloride.

The results of haemoglobin contents also showed slight improvement over the decrease noted after 20 days of treatment. After 20 days of nickel

chloride treatment the Hb % declined by 16.08 %, however, after 30 days it was just 8.92% (Table 27). The significant increase was observed in total WBC counts after separate treatment of the two heavy metal salts for 10, 20 and 30 days exposure. After 30 days of heavy metal exposure, significant depletion was recorded in total WBC count in all the experimental fishes and this depletion was in the order for NiS04 as given in Table 27 and photos in P-39 for Lymphotise in Nickel sulhate . Percent population of macrophages, eosinophills and basophills was found to be increased in all the experimental fishes and the increase was in the order of NiS04 (Table 28). But at the same time the percent population of lymphocytes and monocytes was decreased. Further the increase in macrophages was found to be duration dependent. In Cadmium chloride exposed fish the percent macrophages were 8.39, 10.35 and 13.35 after 10, 20 and 30 days of exposure as against the control values ranging between 2.55% to 2.73%. In the present study the variations in the blood parameters were more in Cadmium chloride than nickel sulphate treated fishes after 10 and 20 days of exposure. The significant increase in RBC count was seen after 10 days of exposure of the fish to sublethal concentration of Cadmium chloride. These results are shone in P-41,42,44,45,46and 47. Number of neutrophls are determined in the P-50,51 and 52, same as the number of Eosinophils are representrd in P-55,56 and 57. Number of Monocytes decrease in *Clarias batrachus* when exposure to 5 mg /l Cadmium chloride in Control after10 and 30 days P-61,62 and 63.

4.3.2 Toxic effect of metals on the blood composition of *Labeo rohita*

Effect of mercuric chloride in haematological parameters. The results are shown in Table: 29. Haematological parameters like total RBC, Hb gm%, and PCV were decreased where as, total WBC counts was increased under these stress of heavy metals under investigation. On mercuric chloride effect uniform reduction in RBC count and Hb content following all

the periods of exposure of the experimental fish (Table 29). In mercuric chloride exposed fishes slight increase in TEC and Hb% was seen after 30 days of treatment. The number of decrease in TEC after 20 days was 2.21 from 2.56 in mercuric chloride exposed fishes, however after 30 days this decrease was just 2.39 from 2.51 (Table 30). The results of haemoglobin contents also showed slight improvement over the decrease noted after 20 days of treatment. After 20 days of mercuric chloride treatment the Hb declined by 5.12 from 6.21, however, after 30 days it was just 5.01 from 5.95 in Table 30. The significant increase was observed in total WBC counts after separate treatment of the heavy metal salts for 10, 20 and 30 days exposure. After 30 days of heavy metal exposure, significant depletion was recorded in total WBC count in all the experimental fishes and this depletion was in the order for HgCl2 as given in Table 29. Percent population of macrophages, eosinophills and basophills was found to be increased in all the experimental fishes and the rice was in the order of HgCl2 (Table 29). But at the same time the percent population of lymphocytes and monocytes was decreased. Further the increase in macrophages was found to be duration dependent. These results are shone in P-40, 43, 48, 49,53and 54. Number of neutrophls are determined in the P-58,59 and 60, Number of Monocytes decrease in *Clarias batrachus* when exposure to 5 mg /l Cadmium chloride in Control after10 and 30 days P-64 and 65.

4.4 Histological Studies:

Histological studies have been carried out to investigate the change in the histological in the histological structure of specific vital organs due to the exposure of lethal and sublethal concentration of heavy metals, cadmium, mercury and nickel. The destruction of the gill epithielial tissue of fish exposed to actually lethal levels of heavy metal salts, has been reported in EIFAC (1983)[50] technical paper. For histological studies the fish affected

by lethal and sublethal concentration were dissected and the organ (gill, liver and kidney) were extirpated and fixed in 20% neutral formaline. After following routine microtechnique, serial sections were cut at 6- 8-micron thickness and stained in haematoxylin and eosin. The effect of heavy metals and mixture were studied on some Dhanapakiam P, Ramasamy VK (2001)[46],haematological and biochemical parameters in fish. *Cyprinus carpio* at sublethal level over the period of 30 days. Heavy metal significantly decreased total RBC count, haemoglobin, heamatocrit (Hct) (except copper after 10 days exposure). The WBC count was increased significantly in all the treated fish. The MCH, MCHC, MVC were increased depending upon the exposure period; declined PVC was noticed at 1% level of significance after 30 days on all the treated fish

For histological change in gill, liver and kidney the catfish, *Clarias batrachus* has been exposed to different concentration of cadmium chloride and nickel sulphate. The *Labeol rohita* was exposed to different concentration of mercuric chloride. The test medium was aerated to avoid any possibility of histopathological changes due to depletion of oxygen. The histopathological lessons were observed in gill, liver and kidney of the fish due to the metallic pollutants. Rana KS et al. (1999)[148]. Studied on the acute short term static bioassays were carried out on *Labeo rohita* with tannery and textile dye industry effluents. The 96-hour LC50 values suggest that tannery effluents were more toxic than textile dye industry effluents. Light microscopic studies exhibited severe histopathological alterations in the liver tissue of the fish exposed to the effluents Govindasamy C et al. (1999)[67]. levels of heavy metal in mussel (*Modiolus metcalfi*) and sediment of Coromandel coast, Bay of Bengal were studied. In mussel and sediment, mean concentrations of Zn and Cu and Cd, Co and Ni were high during the post-monsoon and monsoon seasons respectively. Further, the total mean concentrations were similar and were in the order Zn>Ni>Cu>Cd>Co. Among these five metals, Zn and Ni accumulated more than other metals and Cu, Co and Cd had greater

concentration factor than other metals. Jagadessan G et al. (1999) Studies on the histopathological effects of the three different sub-lethal concentrations of mercury on *Labeo rohita* fingerlings revealed that these metallic salts are capable of changes in its cellular levels in gills leading to the death of the fish. The intoxicated fingerlings again treated with mercury free water (up to 25 days), and recognized its organic constituents to near normal condition. Jagadeesan G Vijayalakshmi S (1999)[50] Alterations in protein metabolism in liver, gills and muscle tissues of the freshwater fish, *Labeo rohita* fingerlings under the influence of mercury toxicant followed by antidote (dimercaprol) treatment, have been ascertained by employing biochemical techniques. Protein content showed a consistent increase in the gill tissues. In the liver and muscle following an initial drop at 24 hours, the increase in protein content was not worthy. Pradhan Bijayendra (1998)[138] Seasonal distribution of trace metals (Co and Ni) in water, zooplankton and seaweeds of Rushikulya and Bahuda was studied. Considerable seasonal variations were noticed in the concentration of these two metals. Co concentrations in zooplankton and seaweeds were found to be greater than that of Ni in both the estuaries. The concentration factor (seaweed/water) and (zooplankton/water) showed that Ni>Co in both the Rushikulya and Bahuda estuaries

4.4.1 Structure of gill

The structure of gill of *Clarias batrachus* and *Labeo rohita* inS control condition appear as normal typical gill. The holobranchs made of two hemibranchs bearing a row of long thin primary gill lamellae are seen. Each primary gill lamella bears numerous secondary gill lamellae, which are placed obliquely to its longitudinal axis. The lamellae are added at the tip of the primary lamellae and the older are situated towards the base.

The actual surface for gaseous exchange is provided by the secondary gill lamellae. Each secondary gill lamella consists of a central vascular layer

formed of a blood capillary and pilaster cells, which remain, covered by the epithelial cells. The epithelial cell lining serve as gas exchange surface Fig.P: 1, P: 2 & P: 6. the capillaries of secondary gill lamellae are also known as gill sinusoids

4.4.1.1 Histological effect in gills of *Clarias batrachus due* to Cadmium chloride:

The gills of *Clarias batrachus* were observed for histological change in them, due to exposure of the fish to different concentration of cadmium chloride for different exposure duration. It has been observed that the response was dose and exposure time dependent.

The primary gill lamella of the gills of control fish has a central cartilaginous core, the epithelial cells lining it and the blood vessels. The secondary gill lamella consists of a layer of flattened epithelial cells, pillar cell, blood spaces and mucous membrane. The initial response to cadmium chloride exposure was mucous secretion. Mucous cell appear as dark stained cells. 24hrs exposure to 1.0 mg/l and 2.0 mg/l cadmium chloride showed alterations in the form of fusion of some secondary gill lamellae along their length and excessive secretion of mucous in the interlamellar space Fig.P: 3. The mucous cell appears vacuolated as hollow spheres. In the increased of 10 days to 2.0 mg/l cadmium chloride, swelling of the tips of secondary gill lamellae was noticed. At the tip of the secondary lamellae Hyperplasia also is noticed mentioned in the Fig P: 4

Fusion of secondary gill lamellae was observed in the fish exposed to further increased concentration of the metal 3.0 mg/l, with the epithelial cells of secondary gill lamella showing necrotic changes Fig. P: 5.

4.4.1.2 Histological effect in gills of *Labeo rohita* due to mercuric chloride:

In *Labeo rohita* was exposed to mercuric chloride in different concentration and different durations to study the histological alternations in the gills. The histological alteration was observed to be dose and duration of exposure dependent.

The first symptom observed in 100-µg/l concentration of mercuric chloride was the excessive secretion of the mucous over the gill filament in the interlamellae spaces. It is the first protective device against the metallic pollutants. Degenerative change in the epithelial cell were observed after 48 hrs , it include hyperplasia, vacuolization of cytoplasm and eccentric position of nuclei. Dilation of blood vessels was also observed Fig. P: 7. The tip of the secondary gill lamella get swell up (oedematous). The partial separation of the epithelial cell from the basement membrane was observed after 96 Fig.P: 8 hrs. Complete and some lyses blood cells were observed in main blood vessels. The primary lamellae were found filled with blood cell Fig. P: 9. In higher concentration of 200 µg /l and increased duration of 10days, the effect was noticed to be necrosis of the epithelial cells of secondary gill lamella, and it found to be fused. After 20 days exposure the severity of the lesions increased, and the secondary gill lamellae showed disintegration of epithelial cells and appear as necked rows of pilaster cells. In 500- µg /l concentration of mercuric chloride for 10 days, hypertrophy of epithelial cells has been observed. Degenerative changes in epithelial cells and hyperaemia was also observed Fig.P: 10. Similar histological changes were observed in still higher concentration of 800 µg /l in 96 hrs. The cell of secondary gill lamellae have been found to be swollen at the tips showing hypertrophy

4.4.1.3 Histological effect in gills of *Clarias batrachus* due to nickel sulphate:

The gills of *Clarias batrachus* were observed for histological change in them, due to exposure of the fish to different concentration of nickel

sulphate for different exposure duration It has been observed that the response was dose and exposure time dependent. Excessive secretion of mucous Fig. P: 11 in the interlamellar spaces had been observed after 24 hrs. and 48 hrs exposure in 1.0, 2.0 and 4.0 mg/l concentration of nickel sulphate. Slightly swollen epithelial lining of the secondary gill lamella at some places was observed and was found completely detached from the core of pillar cells and the capillaries after 20 days. Fig P: 13. The partial seperation of the epitherlial cells from the basement membrane were observed after 10 days in 6.0 mg/l Fig. P: 12

4.4.2 Structure of Liver:

The yellow brown coloured, lobed liver in *Clarias batrachus* is a diagestive gland found attached to the stomach in the anterior part of the body. The normal liver shows the cords of hepatocytes arranged in a radial net like pattern around the central vein Fig. P: 14. The homgenous cytoplasm is present around the central nucleus of hexagonal hepatocytes.

4.4.2.1 Histological effect in liver of *Clarias batrachus* due to Cadmium chloride:

There is no much histological changes occur in the concentration of 0.5 mg/l of cadmium chloride as compare to 2.0 mg/l, which is, occurring in 5 days. Vacuolar degeneration of cytoplasm of the hepatocytes was observed. Haemorrhage in blood capillaries, engorgement of blood cells and hyperaemia was also observed Fig. P: 15, open spaces were found surrounding the lobules. In 3.0 mg/l Cadmium chloride after 20 days exposure severe degenerative change was observed, vacuolar degeneration and eccentric displacement of nuclei in hepatocytes was observed. The most serve effect of cadmium chloride had seen in 6.0-mg/l concentrations where vacuoles were observed in cytoplasm with nuclear degeneration of hepatocytes. Haemorrhage has been noticed at some places shown by blood filled spaces. Fig. P: 16. In 48 hrs

histopathological is more prominent, haemorrhage with degeneration of hepatocytes has been seen.

4.4.2.2 Histological effect in liver of *Labeo rohita* due to mercuric chloride
Histologically the liver of *Labeo rohita* is composed of large and hexagonal shape of cord of hepatocytes with radial arrangement forming mesh like network. Bile canaliculi and sinusoids and found between the hepatocytes. The normal structure of liver is disturbed due the effect of mercuric chloride. In 50 µg /l concentration after 20 days the histopathological change occur in the form of vacuolar degeneration of the cytoplasm were observed. Fig. P: 17. In 200 µg /l mercuric chloride after 5 days exposure, vacuolar degeneration of cytoplasm and eccentric displacement of nuclei of hepatocytes was seen Fig. P: 18. In 400 µg /l the haemorrhage of blood capillaries was observed was observed in 96 hrs. In 600 µg /l mercuric chloride in 96 hrs. engorgement and hyperaemia of blood capillaries and vacuolar degeneration of cytoplasm was noticed Fig. P: 19. In this concentration the histopathological lesions were prominent and degeneration of hepatocytes and haemorrhage has been seen

4.4.2.3 Histological effect in liver of *Clarias batrachus* due to nickel sulphte:
Liver of *Clarias batrachus* is s yellow brown in colour and attached to stomach in anterior part of the body of the control fish. In 1.0 mg/l concentration after 20days exposure the vacuolar degeneration of cytoplasm of hepatocytes was observed Fig. P: 20. In 4.0-mg/l nickel sulphate these changes were observed in 10 days exposure. In 4.0 mg/l nickel sulphate in 10 days exposure the histopathological lesions in the form of severe vacuolar degeneration of cytoplasm and eccentric displacement of nuclei of hepatocytes was observed with haemorrhage of blood capillaries Fig. P: 21. In 6.0 mg/l and 8.0-mg/l concentrations these histopathological lesion became severe in 20 days exposure respectively.

Haemorrhage in blood capillaries and hyperaemia was observed Fig. P: 22 & 23. In 10 days exposure to 10.0 mg/l nickel sulphate the degeneration of hepatocytes was observed and this was more prominent in the hepatocytes nearer to the blood capillaries where haemorrhage seemed to be more severe Fig. P: 24.

4.4.3 Structure of kidney:

Kidney of control fish of *Clarias batrachus* and *Labeo rohita* showed the normal structure. It comprised of uriniferous tubules (nephrone) showing tuft of capillaries forming gromerulus surrounded by Bowman's capsule, a short neck, proximal convoluted tubules and collecting duct. The interstitial tissue is occupied by the actively dividing haemopoietic tissue. The epithelial lining of renal tubule is made of columnar cells with brush boarder, while cuboidal cells line the collecting tubule. The parenchymatous cells and blood cells form the haemopoietic Fig.P: 25.

4.4.3.1 Histological effect in kidney of *Clarias batrachus* due to Cadmium chloride:

At 1.0 mg/l concentration of cadmium chloride, in convoluted tubule of kidney hypertrophy of epithelial cells has been notice in 96 hrs. Fig.: 26 In 2.0 mg/l concentrations after 10days vacuolization in the epithelial cell was observed with some haemorrhage as shown by some blood spaces Fig.P: 27. Vacuolization and degeneration and empty space is developed in the kidney's tissue of fish in 4.0 mg/l cadmium chloride after 5 days. In 3.0 mg/l same change is seen in the up to 20days. Necrosis of haemopoietic tissue, haemorrhage and degeneration of epithelial cells is seen in the tubule of kidney is occur in the 4.0 mg/l. of cadmium chloride in 5 days.

4.4.3.2 Histological effect in kidney of *Labeo rohta* due to mercuric chloride:

Histological changes are seen in *Labeo rohita* due to vary with concentration and duration of exposure to mercuric chloride. After 48 hrs of exposure to 100 µg /l of mercuric chloride the hypertrophy of epithelial cells lining the proximal convoluted tubule (P.C.T) has been seen Fig P:29. has been seen Fig P:29.In 200 µg /l concentration of mercuric chloride hyperplasia in epithelial cells and oedematous condition in Bowman's capsule was observed in 96 hrs. Fig. P: 30. In 200 µg/l concentration in 10 days pycnosis of epithelial cells and vacuolar degeneration was noticed. Necrotic changes causing tabulonecrosis, haemopoietic tissues exhibited degeneration, oedema in epithelial cells and fragmentation of haemopoietic tissue were seen in 400 µg /l concentration after 10 days exposure.Fig.P: 31

4.4.3.3 Histological effect in kidney of *Clarias batrachus due* to nickel sulphate:

Nickel sulphate affect in the kidney of *Clarias batrachus* by showing marked degeneration of epithelial cells and degeneration of haemopoietic tissue. In 2.0 mg/l concentration the epithelial cells of the convoluted tubule showed the vacuolar degeneration and haemorrhage in 5 days exposure Fig.32. In concentration of 4.0 gm/l after 10days exposure showed necrosis and pycnosis of epithelial cells of the tubule, and degeneration of haemopoietic tissue was also observed Fig P: 33. In 102 hrs at 8.0-mg/l concentration of nickel sulphate same histopathological changes were seen and more severe in 10days exposure Fig P: 34. The aggregation of blood cells near renal corpuscle indicated the haemorrhage near glomerulus Fig.P: 35. Lesions of haemopoietic tissues were observed and vacuolar degeneration of epithelial cells of tubule was observed.

Chapter – 5

DISCUSSION

DISCUSSION

In the present investigation, the mean rates of cadmium accumulation in the various tissues studied was in the order gills > kidneys > liver > skin > muscle and it may be inferred that the pattern of accumulation of cadmium differs from tissue to tissue. Critical analysis revealed that the rate of accumulation in the various tissues in comparison to their respective controls is influenced by the duration of exposure (Table 2). However, there occurred significant variations in the concentrations of accumulated cadmium in the various tissues studied at different exposure periods (Table 3) when compared with the respective preceding exposure periods. This difference in accumulation may be attributed to the proximity of the tissue to the toxicant medium, the physiological state of the tissue, presence of legends having an affinity to cadmium and/or to the role of the tissue in the detoxification process. From the point of view of proximity to toxicant of the various tissues analyzed, gills are in direct contact with the toxic medium, whereas liver and kidneys are exposed through media effect. Even though the gills come into direct contact with the ambient toxicant, the pattern of bioaccumulation showed considerable differences between them (Table 3, Fig. 1). While gill was the organ accumulating the maximum cadmium, skin accumulated a far lower amount. Even though reports indicate a correlation between bioaccumulation of metals and exposure concentration, along with exposure time, GILES, (1988)[41], such a correlation did not exactly seem to fit data from the present study of gill are faced with the same concentration of the toxicant and the same exposure period. Devi Vasundharas Srilatha M, Chitra T (1991)[44] Fish *Clarias* batrachas, Linnaeus were subjected to the specific pollutant; a mixture of $HgCl_2$ and phenol. The LC_{50} concentrations were estimated as $HgCl_2$ 1 ppm and phenol 10 ppm. Control samples were also maintained simuitaneously. Exposure time was maintained upto 120 hrs. The analysis was carried on for the estimation of glucose, cholesterol and protein. No

significant variation was observed in the amount of blood glucose. Pituitary samples showed increase in protein content and decrease in glycogen at 72 hrs.

A probable reason for the observed difference in the metal accumulating capacity of gills may be the physiological state of the tissue and/or structural and functional organization of these organs. In the case of fish and crustaceans, as well as molluscs, gills are one of the target organs to suffer instantaneously from ambient toxicants. One of the basic reasons for the gills to act as the primary site for cadmium accumulation, as observed in the present study, is its external position and its proximity to the ambient toxicants. In addition, the highly branched structural organization of the gill and the resultant highly increased surface area, along with the large volume of water passing through the gill surface and the highly vascular physiological state and the relatively small biomass when compared to their surface area MAYER et al. (1991)[111] make the gill a prime site for cadmium accumulation.

As far as the presence of various legends in the tissues is concerned, being an oxyphilic and sulphophilic element, cadmium undergoes multiple bonding in the body MOORE and RAMAMOORTHY, 1984, forming stable complexes with a variety of organic compounds. In the present study the increased mucogenesis under the influence of toxicant, as also reported by RAJAN and BANARJEE 1991, might result in the formation of a mucous trap over the gills for the Cd2+ ions due to the preferential attraction of cadmium to the -SH groups present in the mucus. The cation binding capacity of the fish mucus is also reported by INGERSOLL et al. 1990. However, according to PAUL and BANERJEE 1997, due to the constant and increased ventilatory movements of the operculam under the influence of the xenobiotics, the protective mucous plug inside the opercular chamber is quite often discharged into the medium. Such discharges of mucous plug might make the gills a more vulnerable site for accumulation of cadmium. All these structural and functional peculiarities

of the gills, along with the high vascularization, might be responsible for the highest rate of accumulation of cadmium.

Nagar RN, Bhattacharya Lata (2001)[124], Impaired testicular function was observed after an exposure of swiss albino mice (30 ± 2 g) to mercuric chloride. A sublethal chronic exposure (0.5 ppm for 21 days) resulted in regressed histological and histochemical properties of the testis. The changes observed were degenerated tunica albuginea, abnormal configurations of seminiferous tubules, deformed primary and secondary spermatocytes, hypertrophy and vacuolization in interstitial cells and Sertoli cells. Rao LM, Ramaneswari (2000)[151], The 96 hr LC50 values of pesticides endosulfan and monocrotophos to *Labeo rohita, Mystus vittatus* and *Channa punctata* have been evaluated. The calculated LC50 values of endosulfan to *Labeo rohita* was 1.404 µg/1, to *Mystus vittatus* was 1.652 µg/1 and to *Channa punctata* was 2.148 µg/l. The calculated LC50 values of monocrotophos to *Labeo rohita* to *Mystus vittatus* and *Channa punctata* were 3.558 µg/l, 2.274 mg/l and 3.285 µg/l respectively.

On the contrary, the skin, which also comes under the direct contact stress of the toxicant, shows a far lesser rate of bioaccumulation. According to RAJAN and BANERJEE 1991 and PAUL and BANERJEE 1996, the mucogenic activity of the body skin epithelium in fish is very high when compared to gills HEMALATHA and BANERJEE, 1997. This increased mucogenesis may play a crucial role in preventing the cadmium ions from entering the body, as the coagulated mucus all over the body might be acting as a protective ion trap. Further, unlike gill, discharge of the body mucus into medium is not an active process as there are no ventilatory movements in the skin epithelium, and the rejected epithelial cells, along with the proteniaceous contents of the other degenerating cells, form a protective scab over the skin. Such a protective covering may act as an efficient trap for the Cd2+ ions and, at a later stage, when the cellular debris along with the mucous mass is released into the medium, the entire accumulated cadmium ions might be rejected into the medium

itself and thereby greatly retard their entry into the body skin. Moreover, the regeneration of the exhausted and sloughed mucous cells is quite quick in the case of body skin when compared to the opercular epithelium PAUL and BANERJEE, 1996 and 1997 and gills HEMALATHA and BANERJEE, 1997, leaving less time for the accumulation of cadmium on the body skin epithelium. The intermittent increases observed in the concentration of cadmium in gill (Table 3) at various stages of exposure may be attributed, at least partially, to the temporary breakdown of the mucogenic barrier due to the exhaustion of the mucous cells after their hyper-activity. Even though liver and kidneys do not come into direct contact with the medium, the cadmium accumulation pattern in them followed more or less the same pattern as that of gills (Table 3). Kidneys are next to gill (Fig. 1) quantity-wise in the accumulation of cadmium. SMITH and BELL 1976, have observed higher rates of heavy metal accumulation, especially in the posterior kidney, a tissue primarily involved in the excretory function. Many other workers have also reported the increased cadmium accumulation capacities of kidneys, liver and gills of aquatic organisms PROTASOWICKI and CHODYNIECKI, 1992; NARAYANAN et al., 1997. One of the main reasons attributed to the increased presence of heavy metals in these organs is their capacity to accumulate cadmium brought by blood from other parts of the body and induce the production of the metal binding protein, metallothionein, which is believed to play a crucial role against the toxic effects of heavy metals by binding them BHATTACHARYA et al., 1985. According to KLAVERCAMP et al. 1984 the gill and the liver, along with kidney, are the main sites of metallothionein production and metal retention. This may be yet another main reason for the enhanced presence of cadmium in the gills, kidneys and liver. In addition, all these tissues are rich in the cadmium binding-SH groups REMA and PHILIP, 1997 and therefore it is not surprising that the metal ions are complexed in these organs.

According to KENT 1998 the liver and kidneys are involved in the detoxification and removal of toxic substances circulating in the blood stream. Moreover, liver and kidneys, being the major organs of metabolic activities including detoxification KLAVERCAMP et al., 1984, cadmium might also be transported into these organs from other tissues, including gills for the purpose of subsequent elimination. Such transportation might lead to higher rates of accumulation in these two organs. The possibility of such detoxification/elimination-related mobilization of accumulated cadmium may be one reason for the intermittent reductions in the quantity of accumulated cadmium in gills in (Table 3) at various stages of exposure. Further, according to DORIAN and GATTONE 1992 unbound metals, such as cadmium and mercury, can be reabsorbed by active transport mechanism in the cells of the proximal convoluted tubule, and once they are in the cells they bind to metallothionein, resulting in their accumulation. All these observations justify the possibility of transporting the trace amounts of cadmium from the various tissues to kidneys. Of all the tissues investigated in the present study, the muscle accumulated the lowest level of cadmium, even after 60 days of the experiment (Table 3; Fig. 1). This finding confirmed the existing reports PROTASOWICKI and CHODYNIECKI, 1992; BARBER and SHARMA, 1998)[18].

Burton et al.(1972)[27] have been first suggested that heavy metals intoxication in fish is the coagulation of mucous on gill and the damage of respiratory surface, which results in Hypoxia. Thirdly, Gardner and LaRoche (1973)[60] has noticed lesions in the nervous system causing incoordiantion and disruption of various metabolic activities. Cardilhac et al. (1979)[39]. have work out the second route, that it may be due to failure of osmoregulation creating electrolyte imbalance due to its effect on kidney. The heavy metals affect the organism in such a manner that the entire body of the fish experiences complex biochemical sequences. Srivastava (1982)[187] observe the evident that heavy metals in low concentration probably act more through hormone and enzyme

disturbance rather than the other suggested routes while higher concentration may act by producing intense and rapid damage to various system., resulting in hypoxia and electrolyte imbalance. Rao LM, Manjula Sree Patnaik R) (2000)[150] study on the concentration of zinc, lead and cadmium in alimentary tract, gill, muscle, kidney and liver of the fish *Mystus vittatus* from Mehadrigedda stream were determined. Different organs were selected as the affinities of heavy metals vary with different organs. Among the three heavy metals, concentration of zinc was high in all the organs followed by lead and cadmium. The concentration of heavy metals from the natural habitat was also analyzed and there is some evidence that accumulation in different organs is influenced by the variations in pH and it was more in acidic pH.

5.1 Acute toxicity of the metallic pollutants:

According to Spehar et al. (1979)[181] has summarized the varying LC50 value of different metals and other pollutants for different fish for different exposure duration under different condition in the review.

5.1.1 Acute toxicity of cadmium chloride to the fish:

Long-term bioassay test have been worked out using cadmium chloride to white sucker Northern pipe, Coho salmon, Lake trout, Brook trout and Brown trout. In the present short-term bioassay test different concentration of cadmium chloride ranging from 0.5 mg/l to 6.0 mg/l were used with a container having control medium without cadmium. The observation summarized in the Table 3 has been plotted on the graph using Probit analysis APHA (1985)[12], Finnney (1971) and Sprague (1973)[185]. Spehar et al. (1979)[181] have calculated the 96 hrs LC 50 value of cadmium nitrate for mosquito fish Gambusia affnis to range from 0.9 mg/l to 2.6 mg/l in different test conditions with variation in temperature, alkalinity and pH. For cadmium chloride, 48 hrs LC50 values were found to vary from 2.7 mg/l to 64.9 mg/l in different condition for Gold fish, Carassius auratus and

96 hrs LC 50 value were found to vary from 2.13 mg/l to 46..8 mg/l in the above stated conditions. LC50 values for 24 hrs, 48 hrs, 72 hrs and 96 hrs were found to be 4.6 mg/l, 2.85 mg/l, 2.62 mg/l and 2.31 mg/l respectively. It is supported by the finding of Spehar et al. (1979)[181]. The toxicity of metal cause in the death of the fish is not only the metal but its also the due to the its interaction with other conditions mentioned earlier. The metal gets accumulated in the body hampering its body activities. It is who have exposed the fry of *Catla catla*, *Cyprinus carpio* and *Labeo rohita* to 0.4 mg/l, 2.0 mg/l, 10 mg/l, 20 mg/l and 40 mg/l cadmium chloride and observed that 0.4 mg/l and 2.0 mg/l gave the same results regarding survival rates and gross production to that of the control while other concentration affected survival rate and gross production was reduced.

5.1.2 Acute toxicity of mercuric chloride to the fish:

The studies on the effect of mercuric chloride on acute and chronic toxicity of mercuric chloride. The result are summarized in the Table-9 and are represented in the Fig. 22. The mortality was assessed at 24 hrs. intervals. The LC50 for the 24 hrs, 48hrs, 72 hrs and 96hrs. were determined by probit analysis, APHA (1985)[12] Finney (1971)[57] and Sprague (1973)[185] These were found to be 680 µg /l, 533 µg /l, 516 µg /l and 428 µg /l respectively.Verma and Tonk were observed the different LC50 value for the effect of mercuric chloride. They calculated the 96 hrs LC50 value of mercuric chloride for the fresh water fish *Notoptoterus notopterus* to be 440 µg /l. mercuric chloride was found to be very toxic, as 800 µg /l concentration was sufficient to cause 100 percent mortality in 96 hrs while only 1000 µg /l was sufficient enough to kill all the fish in 48 hrs. Spehar et (1979)[181] had registered in his review the 96 hrs LC50 of methyl mercury for Japanese medaka to be 88 µg /l while Pandey (1994)[134] recoded 400 µg /l as 96 hrs LC50 for mercury. Mercury gets incorporated into the body organs causing an early damage to the organs and the system, which is evident from the other observation regarding

behavioral changes. In order to record the effects of mercuric chloride on some of the blood parameters of *Anabas testudineus*, fishes were exposed to the various concentrations of mercuric chloride. Total erythrocyte count recorded a decline after an initial increase whereas total leucocyte count a haemoglobin content and haematocrit percentage registered an increase in a dose-dependent fashion. The possible causes of these haematological alterations due to mercury toxicity have been discussed, says by Kumar Kuldeep et al. (1999)[100].

5.1.3 Acute toxicity of nickel sulphate to the fish:

Selvanayagam M, Thatheyus Joseph A (1991)[170], Scale carp fingerlings were exposed to the sublethal concentrations of nickel 2.5.5.0 and 10.0 mg/l and chromium 15, 30 and 60 mg/l for 30 days. The vertebral deformities were observed with the help of radiographs and changes in the vertebral mineral content were also noted Chaudhery and Nath (1985)[32] have studied the nickel toxicity to fish Colisa fasciatus, they proved that hyperglycemia is caused due to nickel. In the present investigation the threshold concentration has been found to be 1.0 mg/l in which first fish died in 96 hrs with maximum 80 percent mortality in 30 days. 30 percent mortality have found at the 2.0 mg/l concentration of nickel sulphate in 30 days. 50 percent mortality was found at 4.0-mg/l concentration of nickel sulphate in 10 days. 80 percent mortality was found at 6.0-mg/l concentration of nickel sulphate in 30 days. 40 percent mortality was found at 6.0-mg/l concentration of nickel sulphate in 96 hrs. 60 percent mortality was found at 6.0-mg/l concentration of nickel sulphate in 10 days. 90 percent mortality was found at 8.0-mg/l concentration of nickel sulphate at the end of 30 days. 70 percent mortality was found at 8.0-mg/l concentration of nickel sulphate in 96 hrs. 80 percent mortality was found at 8.0-mg/l concentration of nickel sulphate in 10 days. Same results of mortality were found to be come in 30 days of 8.0-mg/l concentration of nickel sulphate i.e.. 80 percent. 90 percent mortality was

found at 8.0-mg/l concentration of nickel sulphate in 30 days. All the fish were found to be dead at 10.0-mg/l concentration of nickel sulphate in 20 days. 100 percent mortality was found at 12.0-mg/l concentration of nickel sulphate in 96 hrs. These results are given in the Table—10. Finney (1971)[57], Sprague has introduced the LC50 value of nickel sulphate 11.36 mg/l. in 24 hrs. In 48 hrs LC50 value was calculated to be 8.8 mg/l, in 72 hrs LC50 value was found to be 7.25 mg/l. The trend of the toxicity was similar to those of cadmium and mercury. However, comparatively nickel has been found to be least toxic of the three metals selected for the toxicity test. Ray D, Banerjee SK (Natl Environ Engng Res Inst (1998)[154], Study was made to evaluate the hepatic toxicity of nickel in the fish *Clarias batrachus* exposed to 5,10, 15 and 20 mg/liter of nickel sulfate solution for 30 days. Nickel accumulation increased with increase of concentration and exposure time. The accumulation of nickel was highest in the liver tissue at exposure of 0 mg/liter. The concentrations of malondialdehyde significantly increased in liver, kidney and testes Kallanagoudar YP, Patil HS (1997)[85], The response of the fresh water fish Gambusia affinis to lethal toxicity of copper, nickel and zinc in the water of different hardness (50, 150, and 300 mg/l CaCO3) was investigated. Results revealed that copper was found to be more toxic to male, female and fries than nickel and zinc in all the water hardness. Toxicity of the metals was reduced with the increase in the hardness

McCarty et al. (1978)[113] have attempted to relate the mortality patterns due to cadmium for the gold fish to the chemical changes taking place in relatively soft and hard water. A two- phase mortality pattern in the hard water test indicated the likelihood of atleast two modes of cadmium lethality, enzyme and structural protein level. Devies et al. (1978)[45] determined the acute toxicity of silver to rainbow trout and they have found 96 hrs LC50 value to be 6.5 mg/l in soft water and 13.0 mg/l in hard water. Howarth and Sprague (1978)[76] determined the toxicity of copper over a wide range of combination of water hardness and pH to rainbow trout.

The 96 hrs. LC50 value of total dissolved copper ranged from 20 mg/l in soft acidic water to 250 mg/l in hard alkaline water. The high concentration of these pollutants in fish probably explained their increased mortality rate. Kempf and Sittler (1978)[88] determined mercury and organochlorine compounds in five species of fish following a mass mortality of fish in the Rhine river. The above-mentioned results have been confirmed with many other metals using different fish species and it has established the fact that the toxicity due to heavy metals varies from metal to metals, their concentration as well as pH, hardness, temperature of the water. These finding result observed in the present investigation with cadmium, mercury and nickel were concentration of the metals and exposure time have been found to effect the mortality of the fish, *Labeo rohita* and *Clarias batrachus.*

5.2 Effect of heavy metals on growth of fish:

Effect of heavy metals on the growth of fresh water fish, *Labeo rohita* and *Clarias batrachus* have been studied and the results summarizes in the last chapter.

The fresh water fish *Labeo rohita* showed normal growth in weight and length in the controlled medium by 4.74 and 4.61 respectively in 20days. A gradual decrease in growth was observed when the fish was subjected to mercuric chloride and even the loss of weight was noticed in higher concentration of mercuric chloride of 300 **µg** /l and 400 **µg**/l for 20 days exposure. However, the fish showed different trend in growth in terms of length. The decreasing trend in weight and in length in most of the tests can be explained as the metal ions bound to legend, form complexes and are transported to various tissue. Protein synthesis is affected, protein synthesis in the liver of Cyprinus carpio has been studied by Sharma (1980)[174]. he say that methyl mercury affects and reduce the protein synthesis in liver because of which the growth is affected.

Same as *Clarias batrachus* subjected to cadmium chloride and nickel sulphate showed the similar decreasing trend in growth. In the controlled fish growth in weight and length was to the order of 0.316 and 11.111in one experiment while in the other it was 0.231 and 10 percent weight and length gain respectively. In fish affected by cadmium chloride, with the increase of the metal concentration decrease in growth was observed. It was registered to be 9.185 percent in 1.5 mg/l concentration, which further decreased and registered a loss in weight, while any change in length was not noticed in 1.5 mg/l, 2.0mg/l and 2.5 mg/l concentration of cadmium chloride. The fish affected by nickel sulphate registered a decrease in growth in weight from 0.291 percent in fish treaded by 1.0 mg/l nickel sulphate to 0.093 percent growth in 2.0-mg/l nickel sulphate. A loss in weight was observed in 4.0 mg/l and 6.0-mg/l concentrations in order 0.140 and 0.099 percent while change in length of fish has been 11.76 and 6.25 in 1.0 mg/l and 2.0 mg/l, while further increase in metal concentration caused the growth inhibition in length.

Kiokemeister and Weber (1978)[92] determined the effect of zinc and nickel chloride and their mixtures on their relative growth rate of the juvenile guppy using a two-way classification scheme that showed deferent types of toxicant interaction. The concentration is ranging was 6.36 to 10.8 mg/l of nickel and 0.96 to 2.47 mg/l of zinc. The nickel and zinc dose response curves were not statistically different from parallel in retarding the growth. Weis and Weis (1978)[199] with Killishsh indiacated that while cadmium or methylmercury alone can retard fin regeneration, the effect is diminished when both are present. These results clearly indicate that the metal ions get into the body tissues and interfere with the coenzyme- a function in protein synthesis hampering the growth of the fish.

5.3 Histopathology:

Histopathology studies has been recognized in evaluating the pollution potential of toxicants, since trace levels of these chemicals, when do not

bring about animal mortality over a given period, are capable of inducing considerable organ damage, Kumar and Pant (1984)[102].The histopathological effects of surfactants have been illustrated by Lang (1967)[108] and he has related them to physiological and metabolic changes in his review. Brown et al. (1968)[17]. have reported gill damage by detergents and heavy metals. The histopathology of fish exposed to bleached Kraft pulp mill effluent has been studied by Davis (1974)[42]. Bardach et (1965)[19]. have concluded that the tastebuds in fish were damage by the exposure of detergents. Histopathological studies have been recommended by Wood (1960) for diagnosis of mortalities among fishes. In the present investigation, the histopathology of gills, liver and kidney of *Clarias batrachus* exposed to cadmium chloride and nickel sulphate and of *Labeo rohita* exposed to mercuric chloride have been studied.

5.3.1 Histological changes in the gills due to cadmium, mercury and nickel

According to Roberts (1989)[156] about 60 % area is covered by the gill in the fish and its external location render it the most vulnerable target organ for aquatic pollution. It is because of these reasons that severe histophathological alterations have been noticed in the bronchial tissues of the fish under investigations. The initial response to cadmium, mercury and nickel exposure has been the excessive secretion of mucous, through this response has been observed in exposure to a very low concentration in 100 µg /l in mercury while in higher concentration of 1.0 mg/l and 2.0 mg/l of cadmium sulphate and nickel sulphate. In the higher concentration of the metals mercury, cadmium and nickel the bronchial responses in the form of oedema, hyperplasia and hypertrophy of epithelial cell, swelling at the tips of secondary gill lamella and fusion of secondary gill lamellae were found. Haemorrhage in the blood capillaries and congestions of blood cells in the blood vessels have also been noticed due to prolonged exposure to higher concentration of the metals. Same observations are

noticed in the mercuric chloride experiment to. Salmo gairdneri as reported by Wobeser, G. (1975)[200], puntius sophore reported by Khangarot and Somani (1980)[89] and in Sorotherodon mossambicus reported by Naidu et al.(1983)[125] and methoxyethyl mercuric chloride exposure to Puntius conchonius as reported by Gill et al.1988. The entry of heavy metals from the medium is largely through the gills, Oslon et al. (1978)[133]. It has been explained that different metals have different quantitative effects. They increase the capillary permeability, Robert (1989)54 and Eller (1975)[51]. Meenakshi V et al. (1998)[117]. Study on the sublethal effects of mercuric chloride on the liver glycogen, muscle glycogen, blood glucose and blood lactic acid were estimated in the freshwater fish, *Cyprinus carpio* fingerlings. Fingerlings were exposed to the sublethal concentration of mercuric chloride (3.75 C 10-4 mg/l) for a period of 30 days. Fluctuation observed in the metabolites of glycogen indicates the possible arrival of anaerobic condition of the exposed fingerlings

5.3.2 Histological changes in the liver due to cadmium, mercury and nickel:

Liver is found anterior of the body near stomach, which is yellow in colour. The large hexagonal radially arranged hepatocytes with sinusoids and canaliculi in between, from the normal histological structure of the liver in the fish. The hepatocytes contain homogenous cytoplasm around central nucleus. Kothari Suresh, Bhalerao Sangeeta, Sharma SK (1999)[96]. A hepatoprotective herbal drug, Liv52 was tested against changed in protein contents, alkaline phosphatase and alanine transaminase activities in the liver of a freshwater catfish *Heteropneustes fossilis* (Bloch) challenged with HgCl2. Metal exposure with Liv52 administration brought about changes in protein and enzyme levels to near normal values. Histopathological lesions have been observed in the liver of the fish

subjected to the exposure of heavy metals, cadmium, mercury and nickel. Open spaces around the liver lobules, haemorrhage in blood capillaries, hyperaemia, nuclear degeneration and degeneration of hepatocytes are the histopathological changes observed due to metal treatment.Sastry, K.V. (1979).[165] has reported severe damage and degeneration of hepatocytes due to the effect of cadmium on Heteropneusted fossilis. These histopathological changes, due to the effect of various heavy metals, observed by different workers confirm the findings of the present investigations on the effect of the heavy metals, cadmium, mercury and nickel to the fresh water teleosts, *Clarias batrachus* and *Labeo rohita*.

5.3.3 Histological changes in the kidney due to cadmium mercury and nickel:

Histopathological change occur in the fresh water fishes in kidney due to cadmium, mercury and nickel are hypertrophy of epithelial cells of convoluted epithelial cells, haemorrhage, necrosis of haemopoietic tissue, *tubulonecrosis, oedema in epithelial cells and oedematous conditions in* Bowman's capsule. According to Krishnakumari et al. (1983)[98], kidney is the organ to accumulate high concentration of metals, specially mercury. Kirubagaran, R. and Joy, K. P. (1988)[93] says that in fish it also known as the target organ of pollutants. Wobeser, G. (1975)[200]. reported dilated *Bowman's space and renal tubules in the fingerling of Salmo gairdneri*, injected intraperitoneally with 10.0 µg mercury/Kg body weight after 22 hrs whereas hydropic degeneration of tubular epithelium and scattered spots of tubulonecrosis were exhibited y the fingerlings, treated with 20.0 µg mercury/Kg body weight. Similar responses were observed by Gill et al (1988) in Puntius conchonius after chronic exposure to mercuric and methyl mercury. In the present study the histopathological changes in gills, liver and kidney seem to be almost similar, differing a little, qualitatively and quantitatively. Havu, N. (1969)[72] has reported that the heavy metals cadmium accumulated in the pancreatic islets of cottus scorpius and

selectively damages the β cell, causing impaired secretion of insulin. Vacuolar degeneration of epithelial cells, necrosis, haemorrhage are the common features irrespective of the metals used. However the mercury is the more pathogen then cadmium , and cadmium is high toxic then nickel. Scoot, A.L. and Rogers, W.A. (1980)[169] have reported similar histopathological changes. They have stated that the tissue hypoxia is caused due to the damage of the vascular epithelium of the secondary gill lamellae and similar tissue damage causing hypoxia in liver and kidney cells, producing histopathological lesions.

5.4 Heametology

5.4.1 Haemetological change in the *Clarias batrachus* due to metallic effect:

Various authors have reported accumulation of heavy metals induced tissue damage. Larsson *et al.,* (1980) explained the elevation of RBC count in the titanium dioxide effluent-treated flounders to the release of erythrocyte from the spleen to compensate for an impaired oxygen uptake due to disturbed functioning of the gills. However, initial increase in Hb content in nickel sulphate exposed fishes could be due to catalyzing action of toxicant on the incorporation of the body iron stores into haemoglobin. Nilsson (1983)[130] has suggested that the increase in erythrocytes into the circulation in the fish under stress is because of hyperactivity of chromaffin cells, which release catecholamine, stimulating splenic contraction thereby liberating erythrocytes in circulation. The reduction in RBC count and Hb content after 20 days of exposure indicate anemia associated with erythropenia, similar to earlier reports of Panigrahi *et al.,* (1978)[137] and Agarwal *et al.*, (1983)[1]. The anemia with erythropenia has also been reported earlier in fish after exposure to sublethal dose of nickel, Goel and Gupta, (1985)[65]. The decrease in TEC and Hb content might also be due to failure of renal haemopoiesis. Further various authors have reported accumulation of heavy metals like zinc, copper and nickel in the kidney of

fishes, Andrew, (1992)[8], Rana and Raizada, (2000)[149]; Khunayakari *et al.*, (2001)[91]; Dhanapakian and Ramaswamy, (2001)[47]. Such accumulation might have also affected renal haemopoiesis in the present investigation. Christy (1995)[36] reported damaged RBC in the fish *Catla catla* exposed to potassium dichromate which might have resulted into impaired iron absorption. This might also one of the reasons behind the lower Hb content in the experimental fishes exposed to sublethal doses of the heavy metal salts. Nanda and Behera (1996)[126] reported decrease in total RBC, Hb% and PCV in the fish, *Heteropneustes fossils* after nickel sulphate treatment for 15 days. Haemolysis of erythrocytes was also observed after exposing the erythrocytes, experimentally *in vitro*, to 2mM dichromate for 24 hours, Roche and Boget, (1993)[157]. Ali *et al.*, (2000)2 studied haematological effects of hexavalent chromium in fresh water teleost, *Oreochromis mossambicus* and got the similar results with reduction in Hb and TEC. Further, the fluctuations in the blood parameters (TEC and Hb%) after the treatment with nickel and Cadmium may be due to the disturbances in the metabolic activities of the haemopoietic organs and depressed tissue respiration due to toxic effect of heavy metals. In the stress condition fish exhibits asphyxiation due to respiratory failure and anaerobic glycolysis is enhanced. Thus, these physiological changes suggest prevalence of hypoxic environment in the blood in the treated fishes, which cause haemodilution. In the present investigation, experimental fishes exposed to nickel sulphate, erythrocytosis and increase in Hb content following 30 days of stress after initial decrease was seen (Table 27). This can be due to impairment of gas exchange by the gills and lining of operculum (Larson *et al.*, 1980; Haniffa *et al.*, (1985)[71] and the consequent excitation or stimulation of erythropoiesis or compensatory erythropioesis (Larson *et al.*, 1980). However this type of compensatory reaction usually stimulates erythropioesis, thereby leading to the release of immature erythrocytes into the circulating blood, Joshi, 1989)[83]. Increase in WBC count as in the present study was also reported

by Singh (1995)[177]; Ray and Banerjee (1998)[155]; Ali *et al.,* (2000)[2] in various fresh water fishes exposed to different heavy metal salts, suggest *induction of some pathological effect and this might be due to the effect of* metal toxicants on bone marrow. Leucocytosis has been considered to be an adaptation to meet stressful conditions by animals. This is in agreement with the finding of Hota (1995)[74]. In the present investigation depletion in WBC count was observed after 30 days of exposure in all the *experimental fishes. These results are well in agreement with Gill and* Pant (1987)[12]. The depleted WBC count along with depleted Hb content and RBC count indicates dysfunctioning of haemopioietic systems along with dysleucopoisis. This is most probably due to bone marrow depression and liver dysfunction. The increase in leucocyte number in all the experimental fishes after 10 and 20 days of heavy metal treatment is probably for the removal of cellular debris of necrosed tissue at quicker rate as reported by McLeay and Brown (1974) [116] in *Oncorhynchus kisutch* under the chemical stress. The increase in the number of WBC may *play* important role in immunological *defense systems during exposure to toxicants like heavy metals and appears* to be associated with increased circulatory levels of granulocytes, which are known to respond for phagocytosis, Briton, (1963)[24]. This suggests the development of a certain degree of tolerance during toxicant stress condition. In the experimental fishes the PCV values decreased significantly due to decline in RBC count. Appreciable decline in PCV in the present study reflects the anemic state exposure to toxicants like heavy metals and appears to be associated with increased circulatory levels of granulocytes, which are known to respond for phagocytosis, Briton, (1963)[24]. This suggests the development of a certain degree of tolerance during toxicant stress condition. In the experimental fishes the PCV values decreased significantly due to decline in RBC count. Appreciable decline in PCV in the present study reflects the anaemic state of fish. In the opinion of Johansson-Sjobeck and Larsson (1979)[82] **Larsson** anemia is an early

manifestation of acute and chronic intoxication of heavy metals. Importance of these changes may be understood in terms of oxygen consumption in fish resulting in mass fish kills due to heavy metal pollution. Like other stressors and pollutants, Banerjee, 1986)[5], heavy metals influenced and increased red cell indices (Table 27). Reasons that may be attributed for the anaemic state of fish under the toxic stress of nickel sulphate and Cadmium chloride may be inhibition of erythropoiesis and haemopoietic tissue and haemodilution, Larsson (1975)[109]. In all the experimental fishes increase in the macrophages was observed. This increase is may be attributed to the tissue damage. The increased macrophages could engulf the cell debris and could eliminate them preventing bacterial infection in the damaged tissues. Thus increased population of macrophages is an bio-indicator of tissue damage, but this increase is not appearing to be heavy metal specific, though the severity of increase in macrophages appears to have some correlation with the toxicity level of the metals. But it might have some definite correlation with the degree of tissue damage. protective response during heavy metal exposure. Thus increase in macrophages in DLC can be used, as a diagnostic tool to assess the severity of the toxic effect though is not heavy metal specific or toxicant specific

Such a response could be the result of direct stimulation of the immunological defense or could be associated with the heavy metal erythropenia has also been reported earlier in fish after exposure to sublethal dose of nickel, Goel and Gupta, (1985)[65]. The decrease in TEC and Hb content might also be due to failure of renal haemopoiesis.

5.4.2 Haemetological change in the *Labeo rohita* due to metallic effect:

The importance of haematology in the diagnosis of fish diseases and for the assess-ment of the effects of pollution has been widely accepted (Schumacher ***et al.,*** (1956)[168]; McKim ***et al.,*** (1970)[114]; Christensen ***et al.,*** (1972)[35]; McCarthy ***et al.,*** (1973)[112]; Christensen ***et al.,*** (1978)[34]. However,

to establish these blood tests, a thorough understanding of the ' normal range ' of the blood parameters and their variations with age, sex, activity and the physico-chemical properties of the water is needed. The blood values reported here are within the range found for other fish species. Haematocrit values, haemoglobin concentration and erythrocyte counts are com-parable to the values reported for ***Curussius aurutus*** (Anthony, 1961)[10], ***Merluccius merluccius*** (Conroy & Rodrigues, 1965)[37], ***Anubus testudineus*** (Banerjee, 1966) and ***Cyprinus carpio*** (Fourie & Hattingh, 1976). Lower values in ***C. carpi0*** (Field ***et*** *ul.,* (1943)[55] Murachi, (1959)[122]; Houston & De Wilde, (1971)75 and higher values in ***A. testudineus*** (Dube & Munshi, 1973)[48] and ***Amphipnous cuchiu*** (Mishra ***et*** *al.,* 1977)[119] than those of ***L. rohita*** have been reported. The males showed higher blood values as reported in ***C. carpio*** (Fourie & Hattingh, 1976). Ezzat ***et*** *al.* (1974)[53] also found higher erythrocyte counts in males than in the females of ***Tilapiu zillii.*** This appears to be related to the activity of the sexes, *males being more active, and also appears to be associated with gonadal* activity and the associated endocrine factors. High blood values during the breeding season have been reported in ***T. zillii*** (Ezzat ***et*** *ul.,* 1974)[53] and C. ***carpio*** (Fourie & Hattingh, 1976).

Seasonal variations in blood values appear to be closely related with the temperature of the water, being raised during summer and the monsoon months when the tem-perature of water is high. Umminger & Mahoney (1972)[194] reported a haemoglobin maximum during summer in ***Sulmo gairdneri*** and Ezzat ***et*** *al.* (1974)[53] found highest erythrocyte and leucocyte counts in August in ***T. zillii.*** Total leucocyte counts were high during the winter contrary to the findings of Ezzat ***et*** *al.* (1974)[53] who reported high leucocyte numbers in August.

5.5 Accumulation of metals in the fish:

Several scientists tried to find the correlationship of the element including heavy metals present in the water and the amount of these elements

present in the body of the fish inhabiting that water. According to Anti Susan T, Veeraiah K, Tilak KS (1999)[11]. the three Indian major carps *Labeo rohita, Catla catla and Cirrhinus mrigala* were exposed to sublethal and lethal concentrations of fenvalrate pesticides. It was observed that Catla *catla* and *Labeo rohita* exposed to sublethal concentrations for 10 days, ie 0.0007 mg/l and 0.0011 mg/l respectively accumulated higher concentrations than in lethal concentrations. Whereas, *Cirrhinus mrigala* accumulated more in lethal concentrations (0.006 ppm) than in sublethal concentrations (0.0006 ppm). Davis, J.A. (1978)[41]. analysed 18 species of fresh water fish from lentic waters to determine, if the concentration of element in water significantly affect their composition. The relationship between calcium, magnesium, sodium, potassium, nitrogen, sulpher and zinc concentration in the fish and the water from which they were taken was not found to be significant. Spehar, et al. (1979)[181]. Present study was undertaken to determine the accumulation of the metals in the gills, liver and kidney of the fish *Clarias batrachus* and *Labeo rohita* subjected to different concentration of the metals and different exposure time. Controlled fish do not show the accumulation of metal.

5.5.1 Accumulation of cadmium in gills, liver and kidney of *Clarias batrachus* :

The fish *Clarias batrachus* were treated with varying concentration of cadmium chloride ranging from 0.5 mg/l, 1.0 mg/l, 2.0 mg/l, to 5.0 mg/l. the fish were sacrificed at 24 hrs, 48 hrs, 96 hrs and after 30 days, however in 5.0 mg/l the fish did not survive after 5 days, when the fish were dissected and the organs gills, liver and kidney were extirpated and preserved for estimation of accumulation of metal n those organs. In 0.5 mg/l cadmium chloride the accumulation in gills was found to be 3.6, 3.8 and 10 percent in 48 hrs, 96 hrs and 30 days exposure. Accumulation of metal was not detected in 24 hrs in the gill, liver and kidney. In liver and kidney 1.2, 1.5 and 3.0 percent and 0.4, 0.8 and 1.2 percent of accumulation are found

respectively in 48 hrs, 96 hrs and 30 days. In 1.0 mg/l the accumulation of cadmium in gills was 3.8, 4.2, 4.8 and 11.6 percent in 24 hrs, 48 hrs, 96 hrs and 30 days while in the same exposure period it was in liver to be 1.4, 1.75, 1.9 and 3.6 percent and in kidney 0.5, 0.85 and 1.9 percent in 48 hrs, 96 hrs and 30 days. In kidney the accumulation in gills in the same exposure periods was found to be 3.9, 4.45, 5.1 and 12.2 percent, while liver registered the accumulation of 1.5, 1.9,2.3 and 3.8 percent. In kidney 24 hrs exposure did not registered any accumulation in 2.0 mg/l concentration while 0.7, 0.9 and 2.0 percent accumulation were recorded in kidney. The maximum accumulation was estimated in 5.0-mg/l concentrations where the fish survived for 5 days only when it accumulated the maximum of 12.8 percent in gills, 4.25 percent in liver and 2.3 percent in kidney. Gajewskai (1978)[58] observed cadmium content in fresh water fish netted in several polish river system and found the cadmium content to be ranging from 0.01 ug/l to 0.144 ug/l in tissue, much below the allowable limits of 400 to 500 mg cadmium per person per week dose recommended by FAO/WHO Expert committee. Bohn and Fallis (1978)[22] detected the range of cadmium upto 2.3 mg/l in liver. Exposure to 2.5 mg/l in contaminated lake resulted in the accumulation of whole body burdens of 3.19 mg/l in 12 hrs, while the same level was accumulated in 8 days in the laboratory exposure to 48 mg/l. Borg and Lithner (1978)[23] have investigated the downstream from a nickel cadmium accumulator factory and reported the increased levels of cadmium and nickel. Ruparella et al.(1992) reported the accumulation of cadmium and biochemical alteration in the liver of *Sorotherodon mossambicus* (Peters), at 0.1 , 1.0 and 10.0 mg/l concentration for 1.3 and 6 weeks duration, it is also reported that at 1.0 mg/l concentration the accumulation is maximum in 3 weeks. With this exception the above results indicate the accumulation of cadmium in the body of fish is time dependent and dose dependent and the results of the present investigations are in total agreement with them. The accumulation of Cadmium in gill is plotted in the Table 16, while

accumulation of Cadmium in liver the Table 17. and accumulation of Cadmium in kidney is shown in Table 18.

5.5.2 Accumulation of mercury in gills, liver and kidney of *Labeo rohita*:

The accumulation of mercury was studied in the fish *Labeo rohita* by subjecting the fish to different concentration of mercuric chloride ranging from 50 µg /l, 100 µg /l, 200 µg /l, 400 µg /l to 500 µg /l with a control set in which the fish did not record the accumulation of the metals. Mercury has been found to affect the fish, maximum, where in the amount of the metal concentration used was in micrograms. The accumulation too was of the highest order as in the gills in 50- µg /l concentration of mercuric chloride, it was 3.1 percent to 25 percent found in 24 hrs to 30 days respectively. For the kidney the value of minimum percent recorded in 24 hrs is 1.1 percent and maximum is recorded in 30 days is 14.2 percent, while for the liver the minimum percent were obtained in 24 hrs is 1.3 percent and maximum percent is recorded in 30 days was 10.4 percent.

In 100 µg /l of mercuric chloride the lowest percent obtained is in 24 hrs is 5.2 percent and maximum percent obtained is 28.4 percent respectively in gills. While in liver at 100 µg /l concentration of mercuric chloride in 24 hrs recorded the minimum concentration is 3.4 percent and maximum in 30 days was 11.7pervent. In kidney the minimum percent at 100 µg /l were obtained in 24 hrs is 2.3 percent and maximum percent was 5.6 percent. 200- µg /l treatments caused 5.6 to 31 percent accumulation gills, 3.5 to 12.4 percent in liver and 2.1 to 7.3 percent in kidney in 24 hrs to 25 days time. The highest values of accumulation were recorded in 400- µg /l concentrations in which the fish survived only up to 20 days. The accumulation of the metal in gills was determined to be 5.7 percent in 24 hrs and 30.2 percent; the highest value of accumulation in the experiment was estimated in 20 days exposure. For the same period the accumulation was 3.9 and 13.2 percent in the liver and 2.4 and 7.6 percent in kidney. In 500 µg /l too the survival time of the fish was only 20 days the percent is in

24 hrs is 6.0 percent of metal accumulated in the gill while in 20 days 29.1 percent of mercuric chloride were accumulated in the fish. Same as in 500- µg /l concentration of mercuric chloride in 24 hrs and 20 days the percentages of accumulation are 4.0 percent and 13.0 percent respectively. In kidney at 500 µg /l concentration of accumulation of mercuric chloride is, in 24 hrs was 2.6 percent and in 20 days were 7.5 percent. The accumulation of the metal was found to increase with time and concentration of mercuric chloride treatment to the fish. The gills provided the largest surface exposed to the medium and interacted directly with the metal concentration. The accumulation of mercury in gill is plotted in the Table 19, while accumulation of mercury in liver the Table 20. and accumulation of mercury in kidney is shown in Table 21.

Trump, B.F., Jones, R.T. and Sahaphong, S. (1975)[191] says that mercury accumulatied in the body bound to the cell membrane might inhibit enzyme systems. Mercury has the high affinity to sulphydryl groups of protein and hence the enzyme system is affected. Mercuric chloride and cadmium chloride toxicity to fish Channa punctatus was determined by Kulkarni, R.S. and Rafiq Mohd. (1992)[99] who have reported the combination exposure of cadmium and mercury to be more toxic to that of the individual exposure. Kureishy, Tariq W. and D'Silva, C. (1983)[76], reported that the accumulation of cadmium and mercury in fish and mercury accumulation was found to be greater as compared to cadmium.

5.5.3 Accumulation of nickel in gills, liver and kidney

Accumulation of nickel has been studied in *Clarias batrachus* by treating the fish to varying concentration of nickel sulphate from 1.0 mg/l, 4.0 mg/l, and 6.0mg/l to 8.0 mg/l and comparing the accumulation with that of the control. In 1.0 mg/l of nickel sulphate the accumulation in gills was in the gill are order of 1.2, 1.5, 2.1 and 9.5 percent in 24 hrs, 48 hrs and 30 days exposure. Same result are come as 0.5, 0.7, 0.85 and 2.0 percent in liver while in kidney the metal was not detected in this concentration. At 4.0

mg/l the maximum accumulation in 30 days exposure was found to be 12.0 percent in gills. 1.5 percent in kidney and 3.8 percent in liver. In 30 days the value of accumulation of nickel sulphate are 16percent, 4.1 percent and 2.0 percent respectively in gill, live and kidney, in 6.0 mg/l concentration of metallic sulphate. In 8.0 mg/l concentration of nickel sulpahte the accumulation of metal are as fallows 2.75 percent, 0.85 percent gills and liver, in kidney this concentration is not deducted, the maiximum accumulation in 30days are obtained is in gill, liver and kidney at 8.0 mg/l concentration of nickel sulphhate are 16.5 percent in gills, 4.5 percent in liver and 2.6 percent in kidney. From all these observation it was noticed that gills accumulated the maximum and kidney accumulated the minimum with liver accumulating the metal more than kidney but less than gills. The accumulation of the metal has been found to be the most for mercury, lesser for cadmium and least for the nickel in the present investigation. Kureishy, Tariq W. and D'Silva, C. (1983)[107]. has studied the accumulation of mercury and cadmium in Tilapia mossamibica, Perna viridis and Villorita cyprinoids and reported mercury accumulation to be greater as compared to cadmium. Sinha, G.M. Kesh, A.B., Sen Gupta, K. and Das, A.K. (1992)[179]. was studied on the accumulation of cadmium in Anabas testudineus, and finding confirm the present investigations by Ruparella, S.G. Verma, Yogendra, Mehta, N.S. and Rawat, U.M. (1992). They are reported the maximum accumulation of the metal in liver in 1.0 mg/l concentration in 3 weeks in the fish, Sorotherodon mossambicus subjected to 0.1, 1.0, 10.0 mg/l of cadmium for 1, 3 and 6 weeks in alkaline and hardwater, however such trend has not been observed in the present investigation in 5 days in 5.0 mg/l concentration and 1.0 mg/l concentration caused 3.6 percent accumulation in 30 days. The accumulation of nickel in gill is plotted in the Table 22, while accumulation of nickel in liver the Table 24. and accumulation of nickel in kidney is shown in Table 26. Nickel show least accumulation in the organ (gill, liver and kidney) as compared to other two organs mentioned in the studied.

The accumulation of the heavy metals in the fish are as order mercury> cadmium> nickel. Analysis of variance for accumulation of nickel in gills of *Clarias batrachus* in different concentration of nickel sulphate and different exposure is ploted in Table-23. Analysis of variance for accumulation of nickel (%) in liver of *Clarias batrachus* in different concentration of nickel sulphate and different exposure is express in the Table No. 25.

The large-scale accumulation of heavy metals in gills may be due, gill provide the largest surface area told by Pandey A.K. (1994)[134]. which come in the direct contact with the surrounding water medium containing metals, and heavy metals precipitate in the mucous secreted by the gills forming organic constituent of the body. Singh, N. (1984)[178]

5.6 Physiological Changes:

Saraiva (1973)[160] has observed a decreased oxygen consumption of guppies, Lebistes reticulates in the toxic concentration of some heavy *metal salts, such as magnesium chloride, lead nitrate and cadmium* nitrate. The breathing distress has observed by Ellis (1973)[52] and he has described it to be due to clogging of the gills with mucous in addition to direct damage caused by heavy metal ions leading to anoxia and collapse of blood vessel in fish. The nerve impulses to the opercular muscles cause the opercular movement, which shows the respiratory frequency in fish. This heavy metal such as cadmium, mercury and nickel altered in the respiratory frequency. Skidmore and Taveli (1972)[180] recorded increased gill ventillaiton, decreased oxygen tension in the aortic blood, slower rate of oxygen consumption of the fish, rainbow trout, subjected to lethal doses of zink sulphate.

5.7 Conclusion:

It has been observed in the present investigation that the three metallic pollutants, cadmium chloride, mercuric chloride and nickel sulphate have affected the fresh water fish, *Clarias batrachus* and *Labeo rohita*

adversely. The concentration of these metallic pollutants affected the fish in such manner that there was increase in mortality with increase in concentration of the metallic pollutants, mortality also increase with increase the exposure of duration

These metlallic pollutant have been found to affect the adversely. However, these have been an increase in opercular movement in first three days followed by decrease in the rate of opercular movement. In the bioassay test the fish have been affected and mortality has been observed to the dose and time dependent.

Weight and length have been observed to be greatly affected by these metallic pollutants. in weight and length in both reduction is notify in *Clarias batrachus* and *Labeo rohita* when they are subjected to these three metallic pollutants cadmium chloride, mercuric chloride and nickel sulphate. Histological change in the organs gill, liver and kidney of the fish have been studied and found that these organs are affected by the metallic pollutants. During bioaccumulation it has been noticed that the organs gill. Liver and kidney accumulate varying degree of metals depending upon the concentration of the metallic of metals depending upon the concentration of the metallic pollutants affecting and the exposure duration for with the fish have been treated with them.

In fish, the toxicity of cadmium is also well known. Exposure of freshwater fish to cadmium leads to a disturbed calcium metabolism, resulting in hypocalcaemia and anomaly of the bone Therefore, cadmium disrupts calcium homeostasis in fish as well as humans. Although many factors might affect the results of tests of the toxicity of cadmium to aquatic organisms Sprague 1985, water quality criteria can quantitatively take into account only factors for which enough data are available to show that the factor similarly affects the results of tests with a variety of species. Hardness is often thought of as having a major effect on the toxicity of cadmium, although the observed effect may be due to one or more of a number of usually interrelated ions, such as hydroxide, carbonate,

calcium, and magnesium. Hardness is used here as a surrogate for the ions, which affect the results of toxicity, tests on cadmium. The BLM, which quantifies the capacity of metals to bind to the gills of aquatic organisms, can be used to calculate the bioavailable portion of dissolved metals in the water column based on site-specific water quality parameters such as alkalinity, pH and dissolved organic carbon U.S. EPA 1999b.

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TABLES

TABLE -1

Physico Chemical Characteristics of Water.

S/No	Parameters	Means Values	Range
1.	Temperature °C	26	25-27
2.	PH	7.2	6.9-7.3
3.	Deissolved Oxygen (DO)	7.6	6.8-7.8
4.	Alkalinity (mg/l as CaCo3)	134	102-146
5.	Hardness (mg/l as CaCo3)	206	186-238

Physico chemical analysis of water: ***It maintains the standards of the test.*** **The mean values of physico chemical of water are given in the Table-1.**

TABLE-2

Summary of analysis of variance (ANOVA) showing the level of significance in the pattern of bioaccumulation of cadmium in gills, liver, and kidneys of *Clarias batrachus* in various exposure groups (10, 20, 40 and 60 days) of 7 ppm cadmium chloride exposure.

Parameter	*Source*	Sum square (ss)	df	Mean square (ms)	F	P
Gills	Total	2.61	14	-	-	-
	Between groups	2.59	04	0.65 0.002	325	< 0.001
	Within groups	0.02	10		-	-
Liver	Total	1.7	14	-	-	-
	Between groups	1.69	04	0.4225	422. 5	< 0.001
	Within groups	0.01	10	0.001	-	-
Kidneys	Total	0.02	14	-	-	-
	Between groups	0.01	04	0.0015	15	< 0.001
	Within groups	0.01	10	0.001	-	-

TABLE-3

Alterations in the bioaccumulation of cadmium (in ppm) in gills, liver and kidneys of *Clarias batrachus* at various stages of 7 ppm cadmium chloride exposure.

Parameter (Bioaccumulation of Cd in various tissues)	Control	Length of experimental exposure (days)			
		10	20	40	60
Gills	0.00 ± 0.00	1.15 ± 0.04	0.92 ± 0.05	0.75 ± 0.01	1.09 ± 0.01
liver	0.00 ± 0.00	0.65 ± 0.02	0.78 ± 0.02	0.92 ± 0.01	0.72 ± 0.00
Kidneys	0.00 ± 0.00	0.77 ± 0.01	0.83 ± 0.01	0.63 ± 0.01	0.99 ± 0.01

Table- 4

Results of Bioassay test. Percent mortality observed in *Clarias* batarícus due to different concentration of cadmium chloride in different exposure duration

Concentration mg/l	Exposure duration in Hrs.							
	24	48	72	96	120	240	480	720
0.5	00	10	10	10	20	30	50	60
1.0	10	10	10	20	30	40	60	60
1.5	10	30	30	30	40	60	60	70
2.0	20	30	30	40	60	60	70	70
2.5	20	40	50	60	70	70	70	80
3.0	30	60	60	60	80	80	100	
4.0	40	80	80	90	100			
5.0	60	80	80	90	100			

Table- 5

LC50 values of Cadmium Chloride to *Clarias batrachus* in different exposure durations and lethal threshold concentration.

Exposure durations	Median Lethal concentration LC50 Values. mg/l	Lethal Threshold concentration mg/l
24 hrs.	4.6	
48hrs.	2.85	0.5
72 hrs.	2.62	
96hrs.	2.31	

Table- 6

Analysis of varience for mortality of *Clarias batrachus* in different concentration of Cadmium chloride and different exposure durations.

Source of variation	Sum of squares	Degree of freedom	Mean squares	F
Between exposure duration	19443.04	7	2777.57	38.45
Between concentrations	46200.00	8	5775.00	79.96
Residual	4044.46	56.	72.22	-

TABLE—7

Results of Bioassay test. Present mortality observed in *Labeo rohita.* due to different concentration of mercuric chloride in different exposure duration.

Concentratin µg/l	Exposure during in hrs.									
	24	48	72	96	120	240	480	552	600	720
50	00	00	00	10	10	10	20	20	20	20
100	00	00	00	10	10	20	50	80	80	100
200	10	10	10	20	40	50	70	80	100	
300	10	20	20	20	40	60	90	100		
400	20	30	30	40	60	70	100			
500	30	40	40	60	80	80	100			
600	40	60	80	90	90	100				
700	60	80	90	100						
800	70	90	90	100						
1000	80	100								

TABLE-8

LC50 value of mercuric chloride to *Labeo rohita* in different exposure duration and lethal threshold concentration

Exposure durations	Median Lethal concentration LC50 Values. µg/l	Lethal Threshold concentration µg /l
24 hrs.	680	
48hrs.	533	
72 hrs.	516	
96hrs.	528	50

TABLE—9

Analysis of varience for mortality of *Labeo rohita* in different concentration of mercuric chloride and different exposure duration.

Source of variation	Sum of squares	Degree of freedom	Means squares	F
Between exposure duration	42489	9	4721	18.38
Between concentrations	74189	9	8243.22	32.09
Residual	20801	81	256.80	-

TABLE –10

Results of Bioassay test. Percent mortality observed in *Clarias* batariCus due to different concentration of nickel sulphate in different exposure duration.

Concentration mg/l	Exposure duration in hrs.							
	24	48	72	96	120	240	480	720
1.0	00	00	00	10	10	20	30	30
2.0	00	00	00	10	20	30	30	30
4.0	10	20	20	30	30	50	60	70
6.0	20	30	30	40	40	60	70	80
8.0	20	40	60	70	70	80	80	90
10.0	40	60	70	80	80	100		
12.0	60	70	90	100				

TABLE-11

LC50 Values of nickel sulphate to *Clarias batrachus* in different exposureduration and lethal thresold concentratin

Exposure duration	Median Lethal concentration LC50 values. mg/l	Lethal Threshold Concentration mg/l
24 hrs	**11.36**	
48 hrs	**8.8**	
72 hrs	**7.25**	
96 hrs	***6.6***	1.0

TABLE-12

Analysis of varience for mortality of *Clarias batrachus* in different concentration of nickel sulphate and different exposure duration.

Between exposure duration	Sum of squares	Degree of freedom	Mean squares	F
Between exposure duration	15421.4	7	2203.05	40.43***
Between concentration	43535.71	6	7255.95	133.16
Residual	2288.6	42	54.49	-

TABLE-13

Change observed in growth (weight and length) of *Clarias batrachus* in different concentration cadmium chloride in 720 hrs. Exposure durations

Concentration mg/l	Total weight or Fish(g)		Percent gain in weight	Percent loss in weight	Total length of Fish(cm)		Percent gain in length	Percent loss in length
	At the Beg.	At the End			At the Beg.	At the End.		
0.00	58.500	58.685	0.316	-	18	20	11.111	-
0.5	53.940	57.102	5.862	-	16	18	12.5	-
1.0	53.650	53.700	0.093	-	16	17	6.25	-
1.5	48.250	52.682	9.185	-	16	18	Not traceable	
2.0	57.542	57.485	-	0.099	16	17	Not traceable	-
2.5	58.240	58.162	-	0.133	17	19	Not traceable	-

TABLE-14

Change observed in growth (weight and length) of *Labeo rohita* in different concentration mercuric chloride in 720 hrs. exposure durations

Concentration µg /l	Total weight or Fish(g)		Percent gain in weight	Percent loss in weight	Total length of Fish(mm)		Percent gain in length	Percent loss in length
	At the Beg.	At the End			At the Beg.	At the End.		
0.00	6.284	6.582	4.74	-	65	68	4.61	-
50	6.045	6.256	3.49	-	63	65	3.17	-
100	7.428	7.554	1.69	-	67	69	2.98	-
200	7.584	7.682	1.29	-	65	67	3.07	-
300	6.542	6.485	-	0.87	70	72	2.85	-
400	6.240	5.962	-	4.45	63	65	3.17	-

TABLE-15

Change observed in growth (weight and length) of *Clarias batrachus* in different concentration nickel sulphate in 720 hrs. Exposure durations

Concentration mg/l	Total weight or Fish(g)		Percent gain in weight	Percent loss in weight	Total length of Fish(cm)		Percent gain in length	Percent loss in length
	At the Beg.	At the End			At the Beg.	At the End.		
0.00	58.250	58.385	0.231	-	20	22	10	-
1.0	52.150	52.302	0.291	-	17	19	11.76	-
2.0	53.650	53.700	0.093	-	16	17	6.25	-
4.0	48.250	48.182	-	0.140	18	18	Not traceable	
6.0	57.542	57.485	-	0.099	17	17	Not traceable	-

TABLE-16

Accumulation of Cadmium (%) in gills of *Clarias batrachus* in different concentration of Cadmium chloride and different exposure.

Concentration mg/l	**Exposure duration in hrs.**				
	24	48	96	120	720
0.5	Not detectable	3.6	3.8	-	10.0
1.0	3.8	4.2	4.8	-	11.6
2.0	3.9	4.45	5.1	-	12.2
5.0	4.05	4.9	6.20	12.8	-

TABLE-17

Accumulation of Cadmium (%) in liver of *Clarias batrachus* in different concentration of Cadmium chloride and different exposure.

Concentration mg/l	**Exposure duration in hrs.**				
	24	48	96	120	720
0.5	Not detectable	1.2	1.5	-	3.0
1.0	1.4	1.75	1.9	-	3.6
2.0	3.9	1.9	2.3	-	3.8
5.0	4.05	2.8	3.2	4.25	-

TABLE-18

Accumulation of Cadmium (%) in kidney of *Clarias batrachus* in different concentration of Cadmium chloride and different exposure.

Concentration mg/l	**Exposure duration in hrs.**				
	24	48	96	120	720
0.5	Not detectable	0.4	0.8	-	1.2
1.0	Not detectable	0.5	0.85	-	1.9
2.0	Not detectable	0.7	0.9	-	2.0
5.0	0.5	1.0	1.2	2.3	-

TABLE-19

Accumulation of mercury (%) in gills of *Labeo rohita* in different concentration of mercuric chloride and different exposure.

Concentration µg/l	Exposure duration in hrs.					
	24	48	96	480	600	720
50	3.1	6.0	7.2	-	-	25
100	5.2	8.6	9.6	-	-	28.4
200	5.6	8.2	9.6	-	31.0	-
400	5.7	10.0	20.1	30.2	-	-
500	6.0	11.1	22.3	29.1	-	-

TABLE-20

Accumulation of mercury (%) in liver of *Labeo rohita* in different concentration of mercuric chloride and different exposure.

Concentration µg/l	Exposure duration in hrs.					
	24	48	96	480	600	720
50	1.3	3.8	5.4	-	-	10.4
100	3.4	4.6	5.6	-	-	11.7
200	3.5	3.9	6.2	-	12.4	-
400	3.9	6.0	9.0	13.2	-	-
500	4.0	7.2	10	13	-	-

TABLE-21

Accumulation of mercury (%) in kidney of *Labeo rohita* in different concentration of mercuric chloride and different exposure.

Concentration µg/l	Exposure duration in hrs.					
	24	48	96	480	600	720
50	1.1	2.3	2.9	-	-	14.2
100	2.3	2.4	3.2	-	-	5.6
200	2.1	2.4	3.1	-	7.3	-
400	2.4	3.2	4.5	7.6	-	-
500	2.6	3.4	6.8	7.5	-	-

TABLE-22

Accumulation of nickel (%) in gills of *Clarias batrachus* in different concentration of nickel sulphate and different exposure.

Concentration mg/l	**Exposure duration in hrs.**			
	24	48	96	720
1.0	1.2	1.5	2.1	9.5
4.0	1.8	2.1	2.6	12.00
6.0	2.5	3.6	5.5	46.00
8.0	2.75	3.8	9.0	16.50

TABLE-23

Analysis of variance for accumulation of nickel in gills of *Clarias batrachus* in different concentration of nickel sulphate and different exposure

Between of variation	Sum of squares	Degree of freedom	Mean squares	F
Between exposure duration	**334.24**	**3**	**111.41**	**52.06**
Between concentration	**49.74**	**3**	**16.58**	**7.74**
Residual	**19.31**	**9**	**2.14**	**-**

TABLE-24

Accumulation of nickel (%) in liver of *Clarias batrachus* in different concentration of nickel sulphate and different exposure.

Concentration mg/l	**Exposure duration in hrs.**			
	24	48	96	720
1.0	0.5	0.7	0.85	2.0
4.0	0.6	0.95	1.5	3.8
6.0	0.8	1.2	1.6	4.1
8.0	0.85	1.3	1.64	4.5

TABLE-25

Analysis of variance for accumulation of nickel (%) in liver of *Clarias batrachus* in different concentration of nickel sulphate and different exposure

Between of variation	Sum of squares	Degree of freedom	Mean squares	F
Between exposure duration	**20.65**	**3**	**6.88**	**36.22*** **
Between concentration	**2.64**	**3**	**0.88**	**4.63** **
Residual	**1.72**	**9**	**0.19**	**-**

TABLE-26

Accumulation of nickel (%) in kidney of *Clarias batrachus* in different concentration of nickel sulphate and different exposure.

Concentration mg/l	**Exposure duration in hrs.**			
	24	48	96	720
1.0	**Not detectable**	**Not detectable**	**Not detectable**	**Not detectable**
4.0	**Not detectable**	**Not detectable**	0.3	1.5
6.0	**Not detectable**	0.2	0.5	2.0
8.0	**Not detectable**	0.25	0.6	2.6

Table – 27

Alternation in total erythrocytes count (TEC), Hemoglobin (Hb), Total leucocytes counts (TLC), Means corpuscular Hemoglobin concentration (MCHC), Packed cell volume (PVC), following exposure to subleathal concentration Cadmium chloride and Nickel sulphate.

Parameters	Exposure to Heavy Metal Salts								
	Control			CdCl2			NiSO4		
	Period (Days)			Exposure Period (Days)			Exposure Period(Days)		
	10	20	30	10	20	30	10	20	30
Total erythrocytes counts (TEC) (million/mm3)	2.72	2.63	2.62	2.13	2.38	2.46	3.42	1.75	1.63
Hemoglobin (Hb) gm%	5.60	5.72	5.16	5.16	4.80	4.70	6.30	4.22	3.38
Total leucocytes counts (TLC) 10^3 cell/mm^3	6.27	6.30	6.35	7.00	8.24	5.07	8.26	9.32	5.27
Means corpuscular Volume (MCV) cu/μ	136.36	132.07	128.62	158.87	156.57	138.01	160.28	161.30	148.25
Means corpuscular Hemoglobin (MCH)/gm	30.26	32.03	32.06	51.28	55.14	59.64	54.64	57.69	62.99
Means corpuscular Hemoglobin concentration (MCH)%	26.46	24.79	24.55	36.28	41.26	42.75	44.33	54.83	60.70
Packed cell volume (PVC) ml/100ml	50.14	50.07	49.80	44.40	40.00	39.00	41.33	36.33	30.00

Table – 28:

Differential Leucocytes count (DLC) of *Clarias batrachus* of sublethal concentration of heavy metals cadmium chloride and Nickel sulphate

		Differential Leucocytes count (DLC)		
Days of Exposure	Leucocytes	Control	CdCl2	NiSO4
10	Lymphocytes %	30.12	25.48	21.26
	Basophiles %	18.55	23.15	28.54
	Eosinophills %	02.86	05.27	06.04
	Macrophages %	02.55	05.32	08.39
	Neutrophills %	37.68	34.47	30.50
	Monocytes %	06.24	05.68	05.04
20	Lymphocytes %	29.82	25.48	21.26
	Basophiles %	19.05	23.15	28.54
	Eosinophills %	02.62	05.27	06.04
	Macrophages %	02.59	05.32	08.39
	Neutrophills %	39.75	34.47	30.50
	Monocytes %	06.16	05.68	05.04
30	Lymphocytes %	30.08	24.12	16.28
	Basophiles %	18.92	25.36	30.06
	Eosinophills %	02.68	06.15	07.32
	Macrophages %	02.73	07.46	13.35
	Neutrophills %	38.25	31.65	28.96
	Monocytes %	06.20	04.92	03.82

Table – 29:

Differential Leucocytes count (DLC) of *Labeo rohita* of sublethal concentration of heavy metals mercuric chloride.

		Differential Leucocytes count (DLC)	
Days of Exposure	Leucocytes	Control	$HgCl_2$
10	Lymphocytes %	28.12	24.46
	Basophiles %	17.60	22.15
	Eosinophills %	02.70	05.42
	Macrophages %	02.92	05.60
	Neutrophills %	36.70	33.80
	Monocytes %	07.10	06.28
20	Lymphocytes %	27.90	26.30
	Basophiles %	18.52	22.48
	Eosinophills %	02.78	05.96
	Macrophages %	02.82	05.50
	Neutrophills %	38.60	35.57
	Monocytes %	06.06	05.46
30	Lymphocytes %	29.08	23.12
	Basophiles %	17.92	24.36
	Eosinophills %	02.75	06.89
	Macrophages %	02.95	07.06
	Neutrophills %	37.35	30.60
	Monocytes %	07.00	04.10

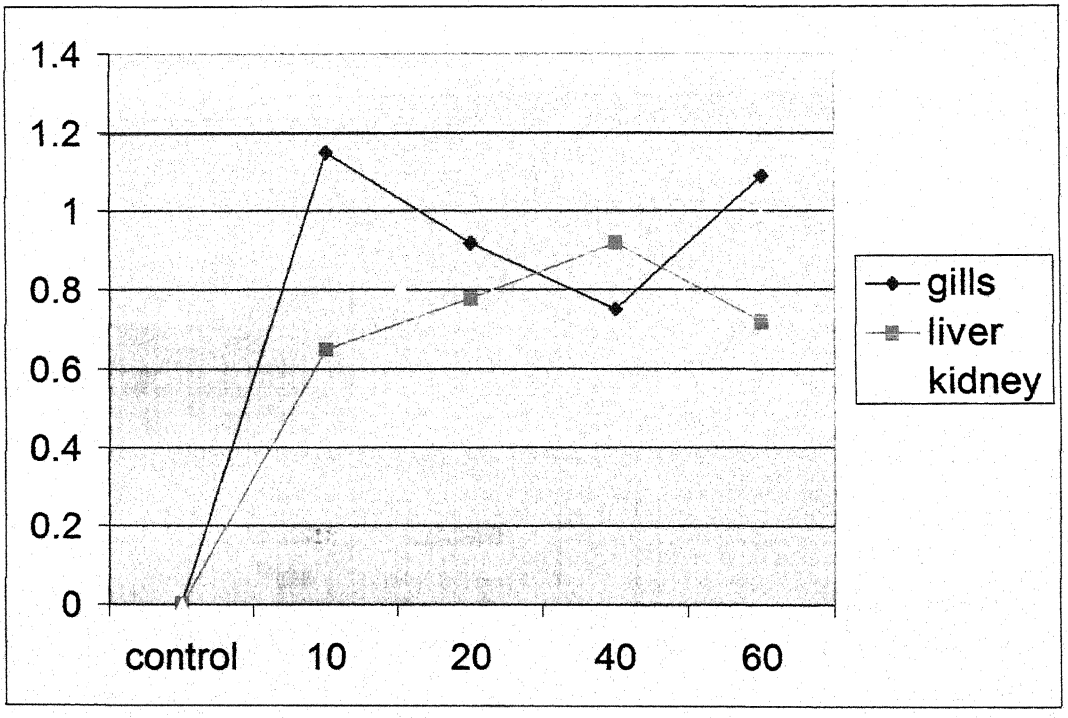
Table – 30

Alternation in total erythrocytes count (TEC), Hemoglobin (Hb), Total leucocytes counts (TLC), Means corpuscular Hemoglobin concentration (MCHC), Packed cell volume (PVC), following exposure to subleathal concentration mercuric chloride.

Parameters	Exposure to heavy metals					
	Control			HgCl2		
	Period (Days)			Exposure Period (Days)		
	10	20	30	10	20	30
Total erythrocytes counts (TEC) (million/mm3)	2.62	2.53	2.51	2.02	2.21	2.39
Hemoglobin (Hb) gm%	6.10	6.21	5.95	5.90	5.12	5.01
Total leucocytes counts (TLC) 10^3 cell/mm^3	6.37	6.41	6.48	7.12	9.04	6.27
Means corpuscular Volume (MCV) cu/μ	140.36	136.05	132.32	168.77	166.66	140.08
Means corpuscular Hemoglobin (MCH) gm	32.23	34.02	34.07	56.18	60.13	64.63
Means corpuscular Hemoglobin concentration (MCH)%	24.41	22.68	22.46	34.80	39.91	40.05
Packed cell volume (PVC) ml/100ml	51.47	51.40	50.90	42.98	36.05	35.0

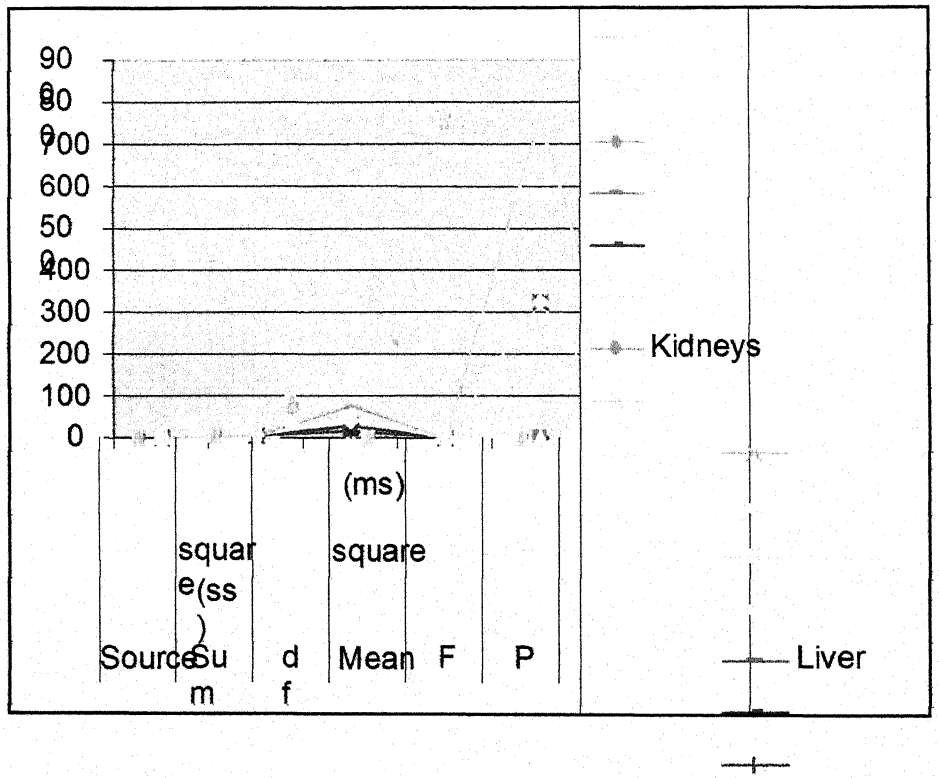
FIGURES

Figure - 1



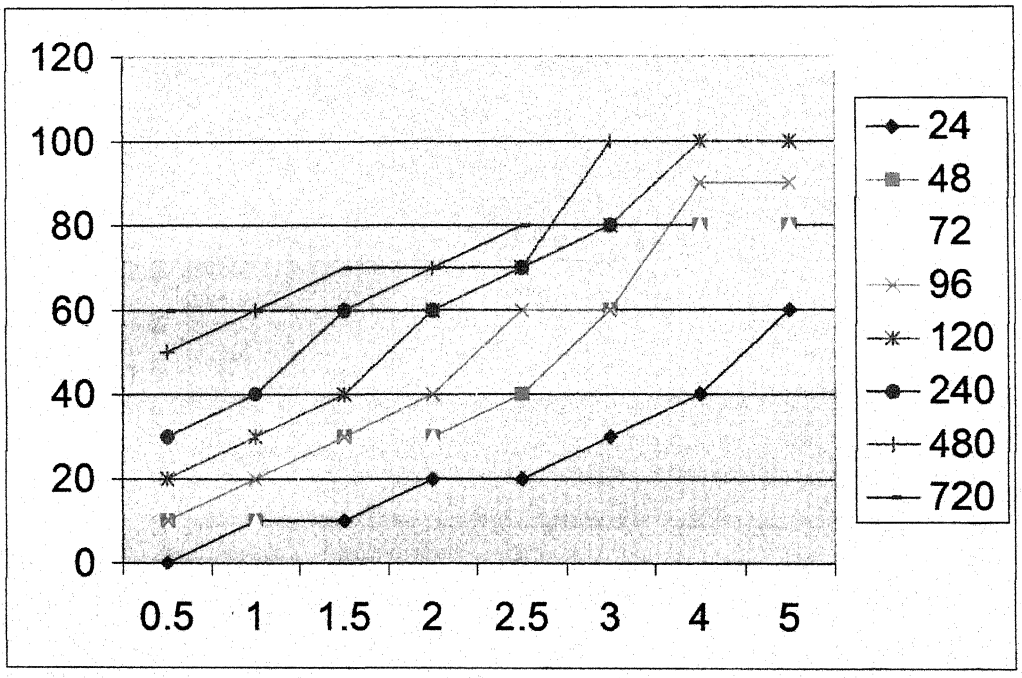
Mean rate of bioaccumulation of cadmium (in ppm) in gill, liver and kidneys of *Clarias batrachus* during 7 ppm cadmium chloride exposure

Figure -2



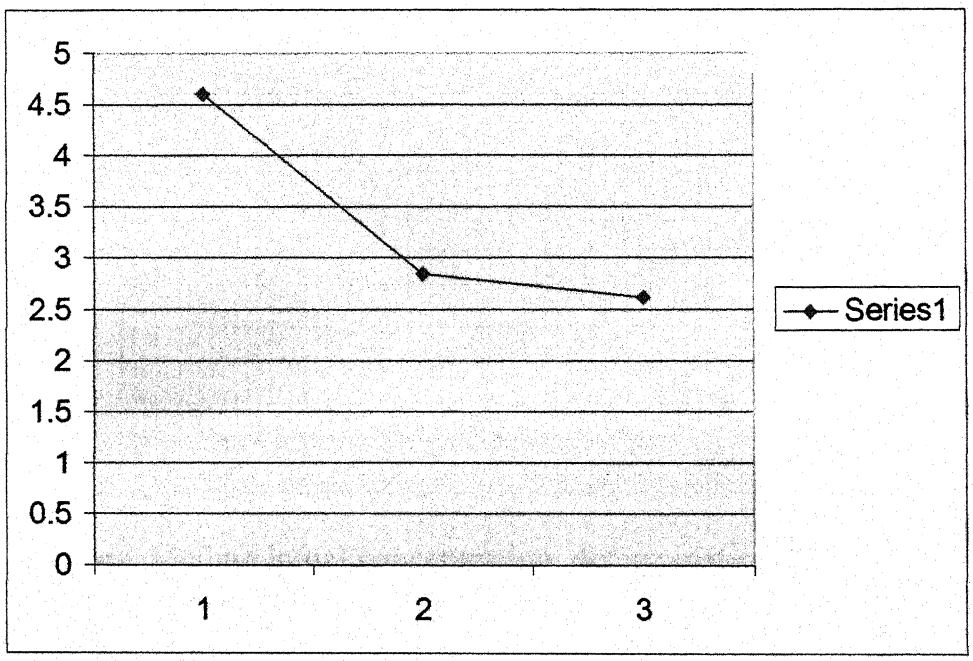
Bioaccumulation of cadmium in gills, liver, and kidneys of *Clarias batrachus* in various exposure groups (10, 20, 40 and 60 days) of 7 ppm cadmium chloride exposure

Figure -3



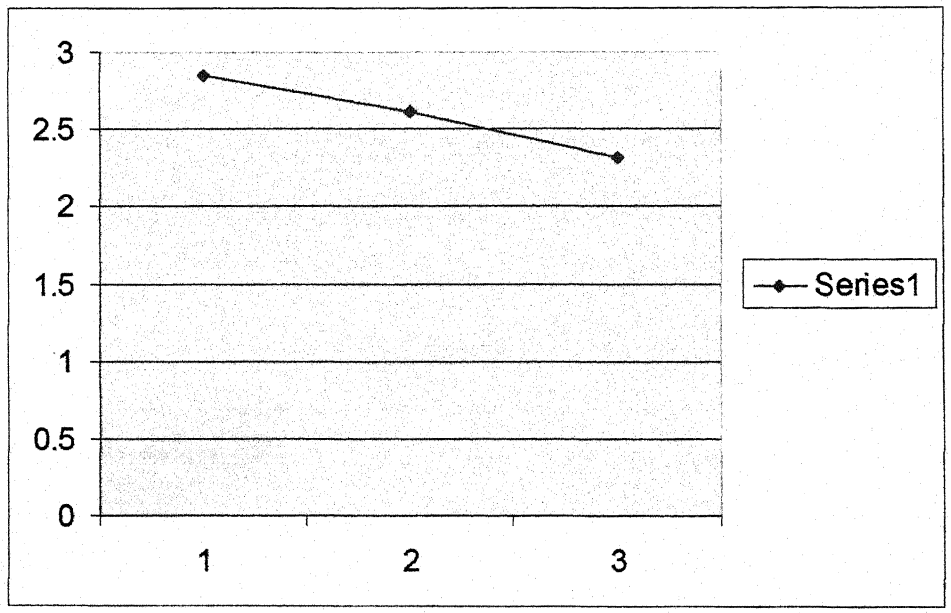
mortality (%) of *Clarias batrachus* in different concentration of Cadmium Chloride in different exposure duration in hrs.

Figure -4



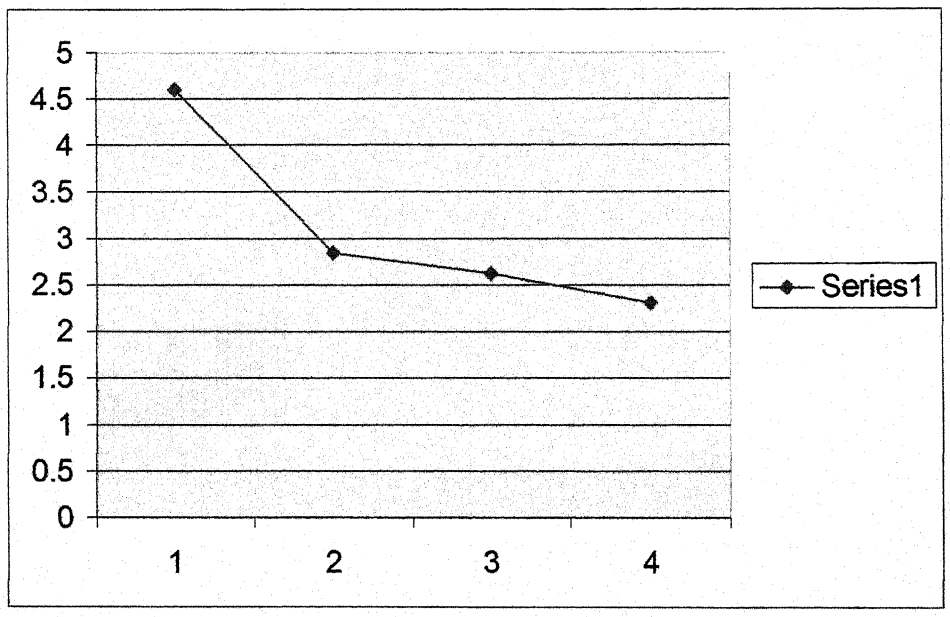
24 hrs. Median lethal concentration determination of cadmium chloride to *Clarias batrachus* by Probit Analysis

Figure –5



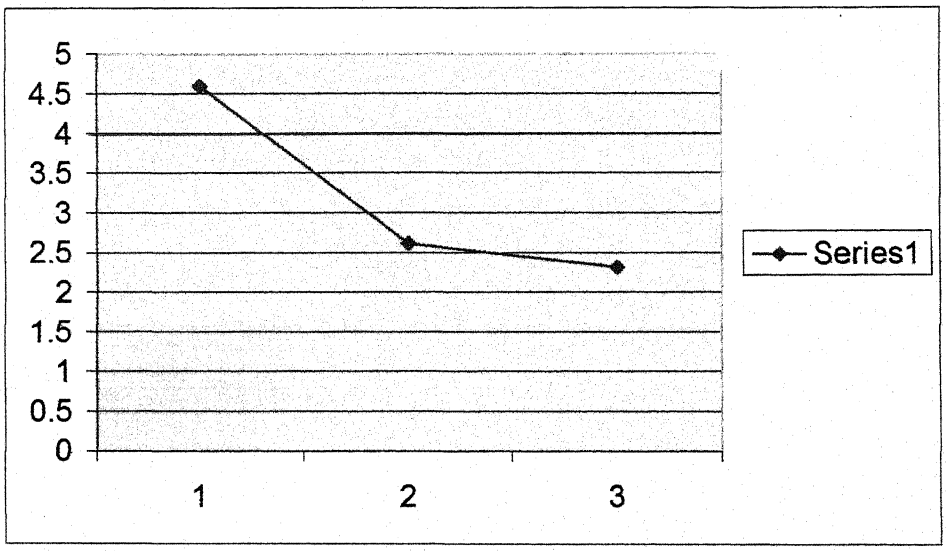
48 hrs. Median lethal concentration determination of cadmium chloride to *Clarias batrachus* by Probit Analysis

Figure -6



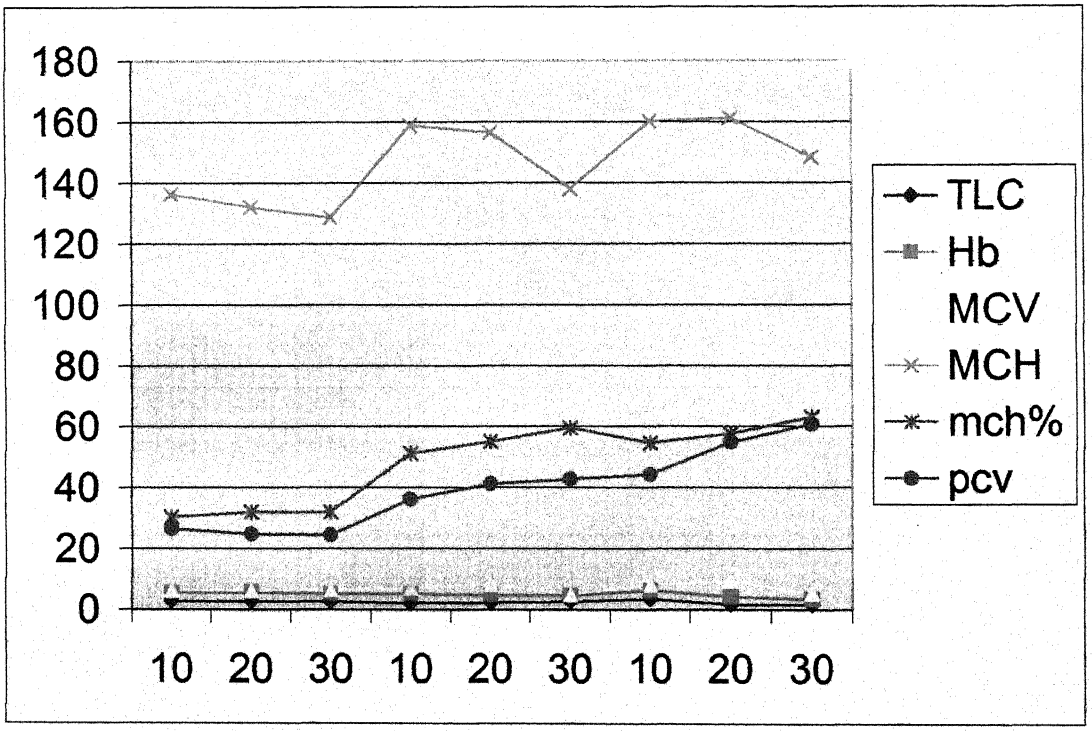
72 hrs. Median lethal concentration determination of cadmium chloride to *Clarias batrachus* by Probit Analysis

Figure -7



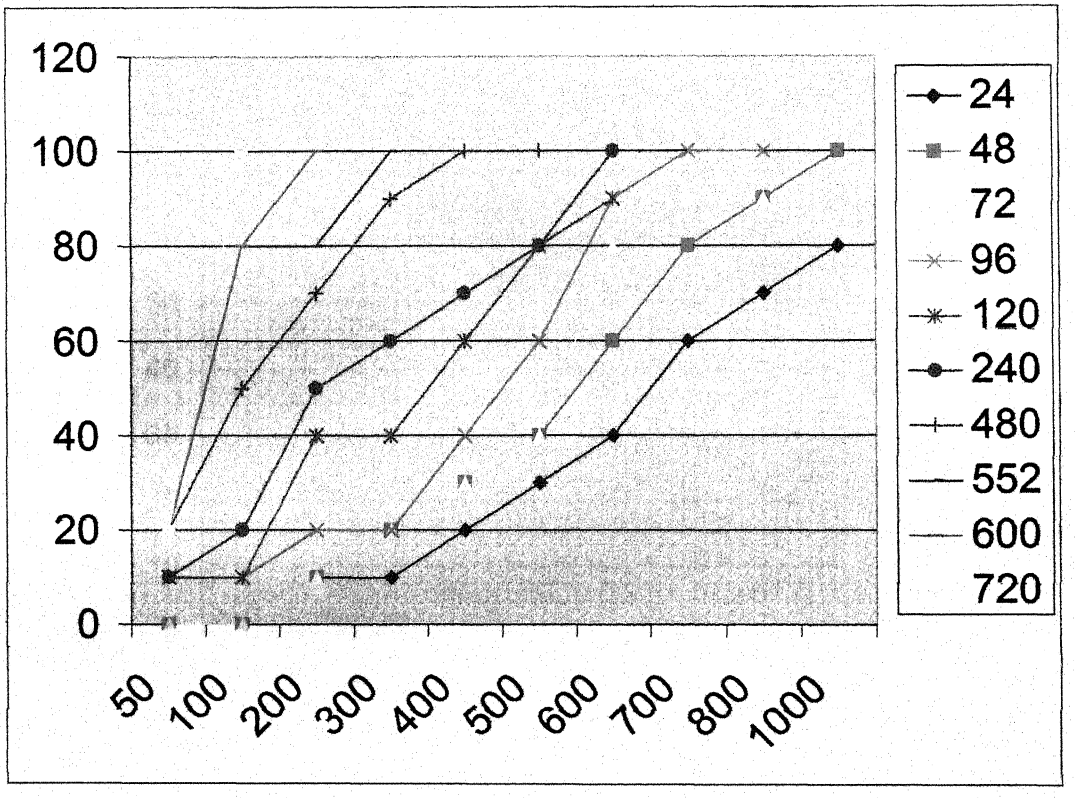
96hrs. Median lethal concentration determination of cadmium chloride to *Clarias batrachus* by Probit Analysis

Figure - 8



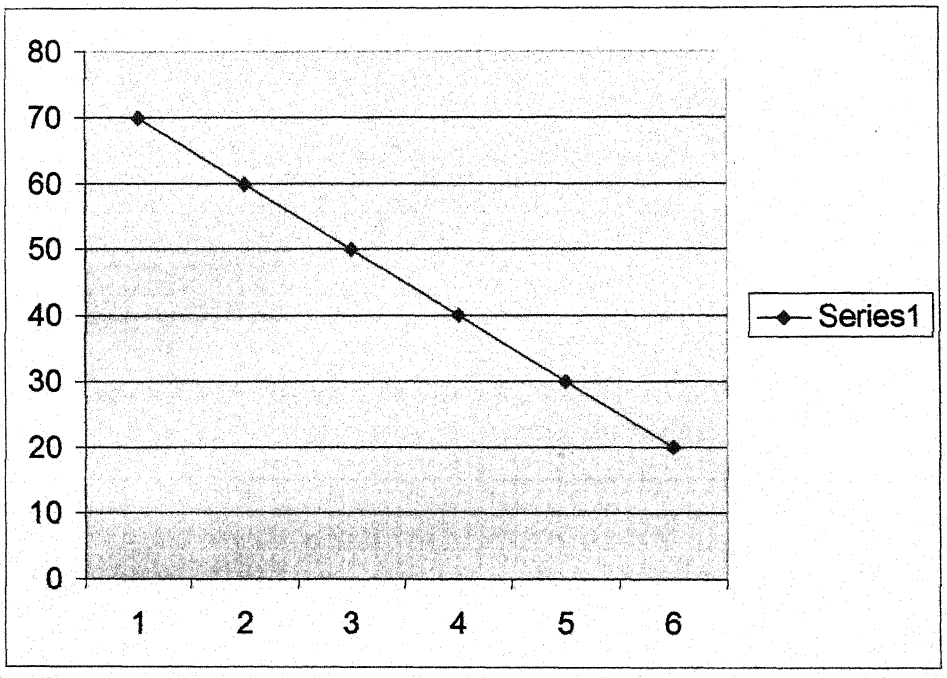
Alteration in the total erythrocyte count (TLC), Heamoglobin(Hb), Total leucocyte count (TLC), Mean Corpuscular concentration (MCHC), Packet cell Volume (PCV); following exposure to subleathal concentration of $NiSO_4$ and $CdCl_2$

Figure -9



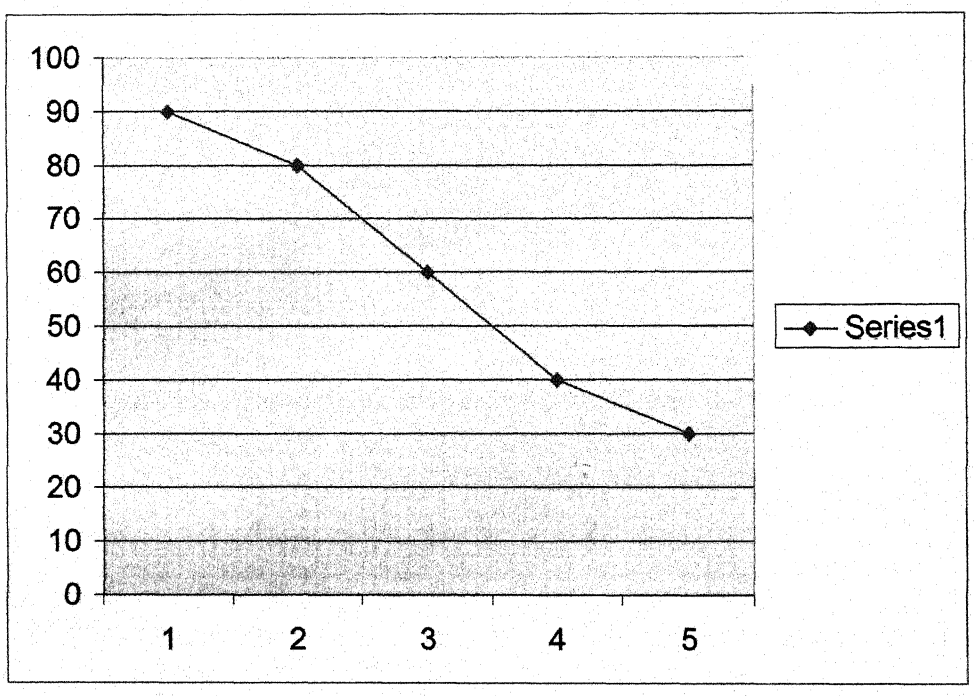
Mortality of *Labeo rohita* due to different concentration of mercuric chloride .

Figure -10



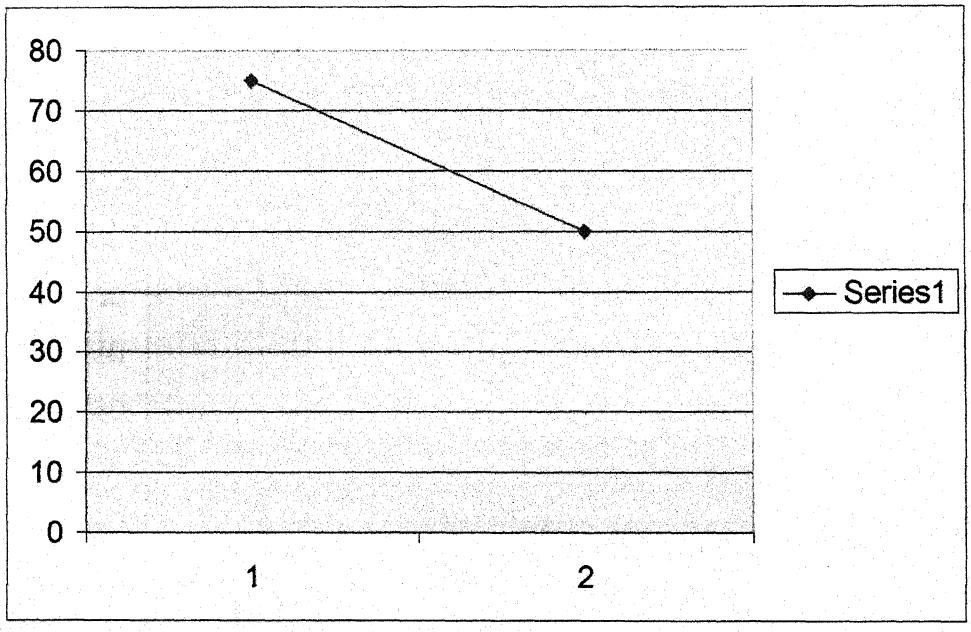
24hrs. Median lethal concentration determination of mercuric chloride to *Labeo rohita* by Probit Analysis

Figure -11



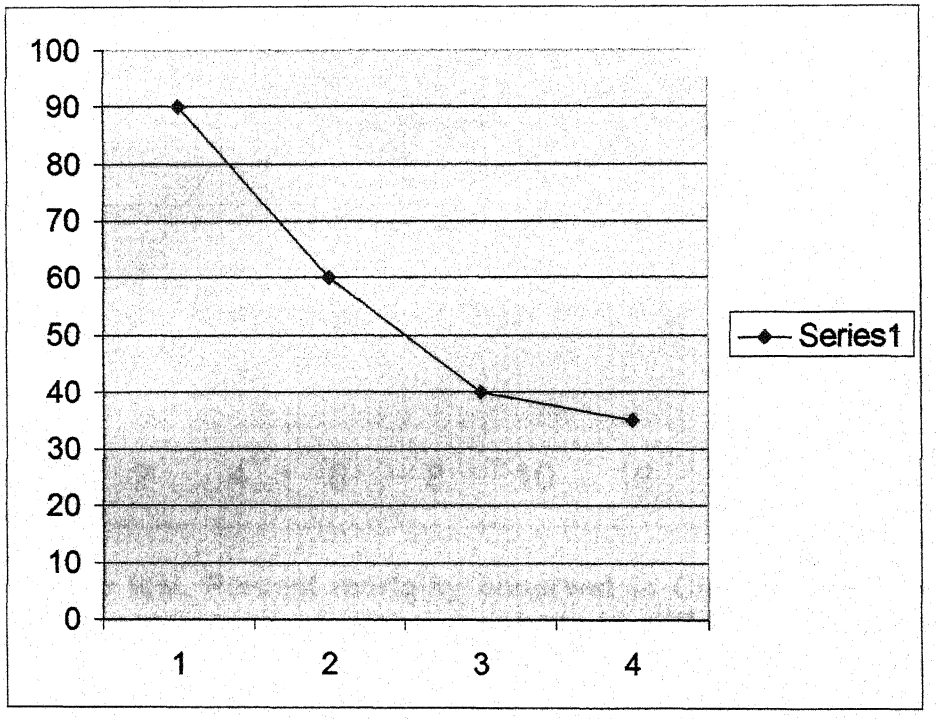
48hrs. Median lethal concentration determination of mercuric chloride to *Labeo rohita* by Probit Analysis

Figure -12



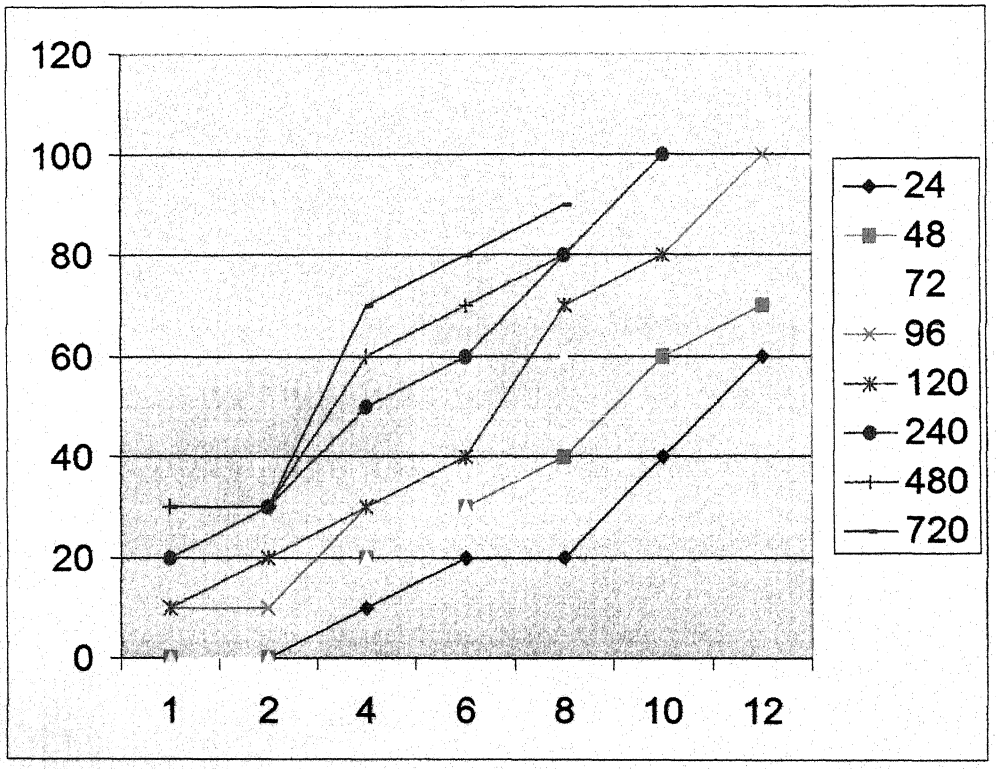
72hrs. Median lethal concentration determination of mercuric chloride to *Labeo rohita* by Probit Analysis

Figure -13



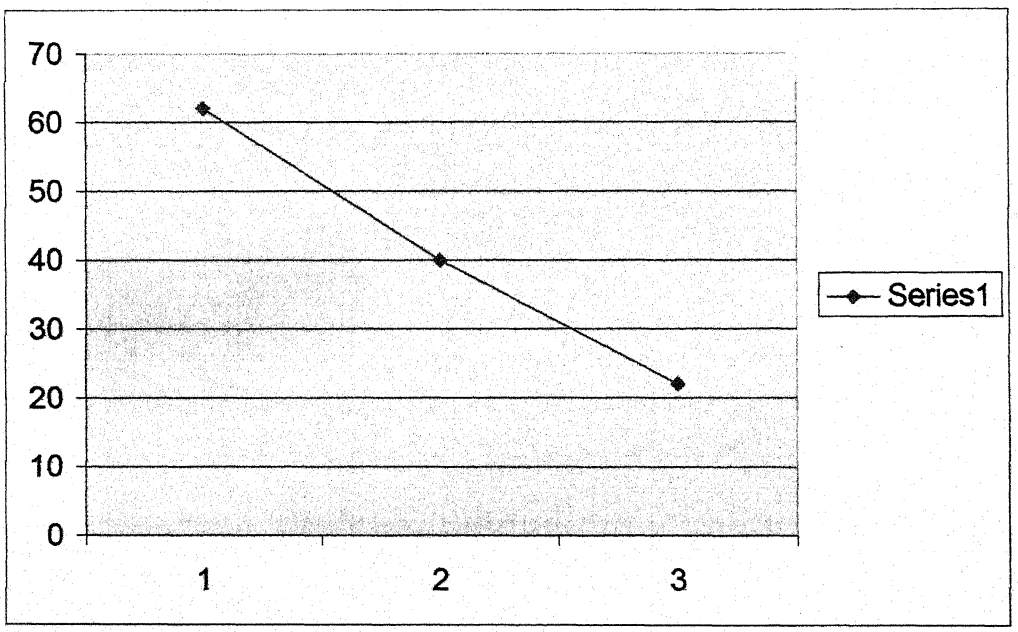
96hrs. Median lethal concentration determination of mercuric chloride to *Labeo rohita* by Probit Analysis

Figure -14



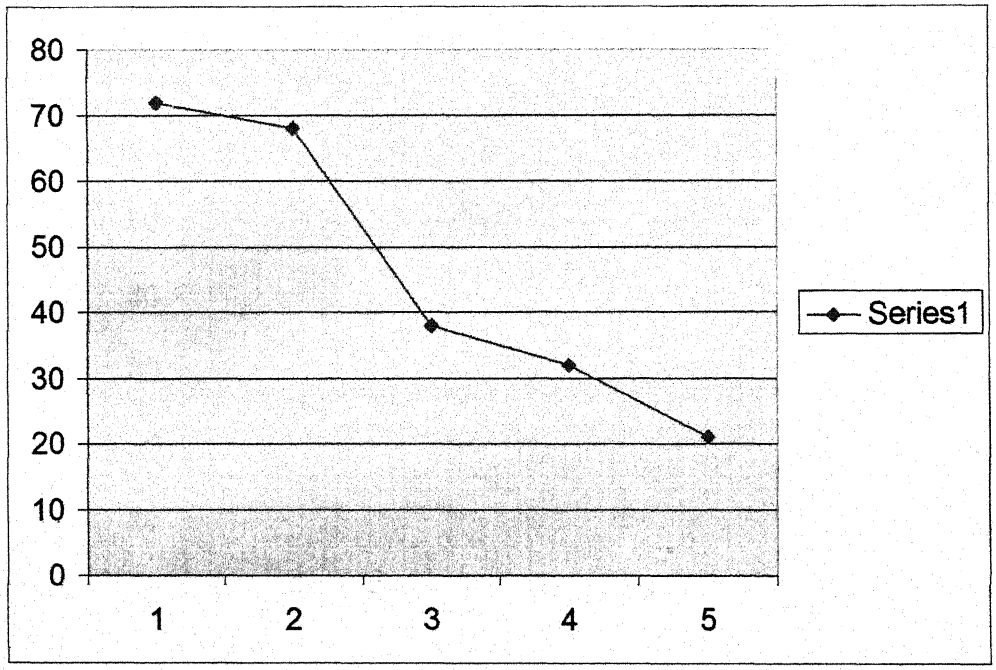
Results of Bioassay test. Percent mortality observed in *Clarias* batariicus due to different concentration of nickel sulphate in different exposure duration

Figure -15



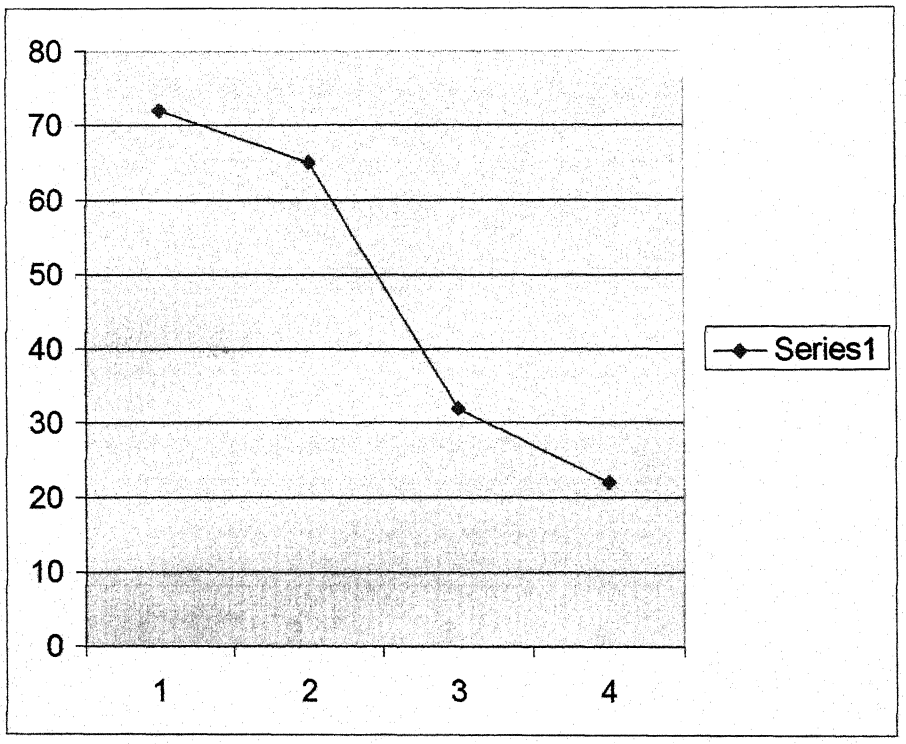
24hrs. Median lethal concentration determination of nickel shalphate to *Clarias batrachus* by Probit Analysis

Figure -16



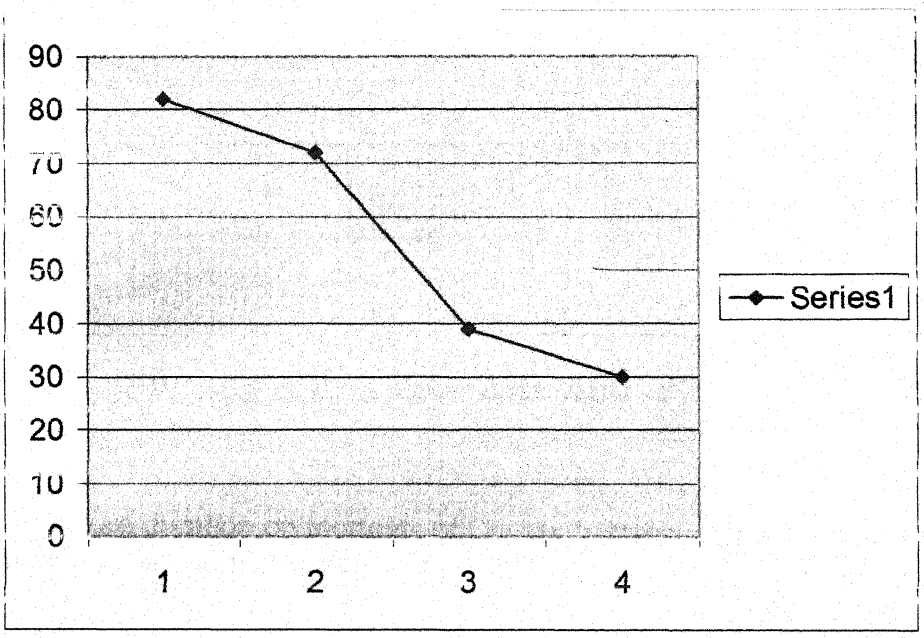
48 hrs. Median lethal concentration determination of nickel sulpate to *Clarias batrachus* by Probit Analysis

Figure –17



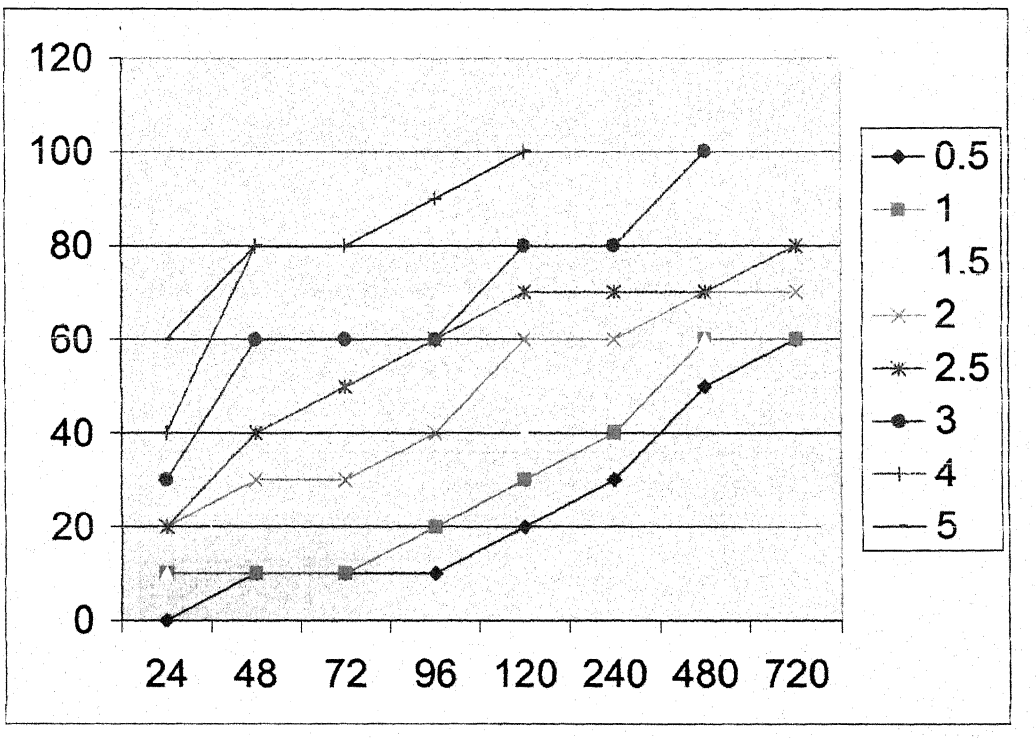
72 hrs. Median lethal concentration determination nickel sulphate to *Clarias batrachus* by Probit Analysis

Figure -18



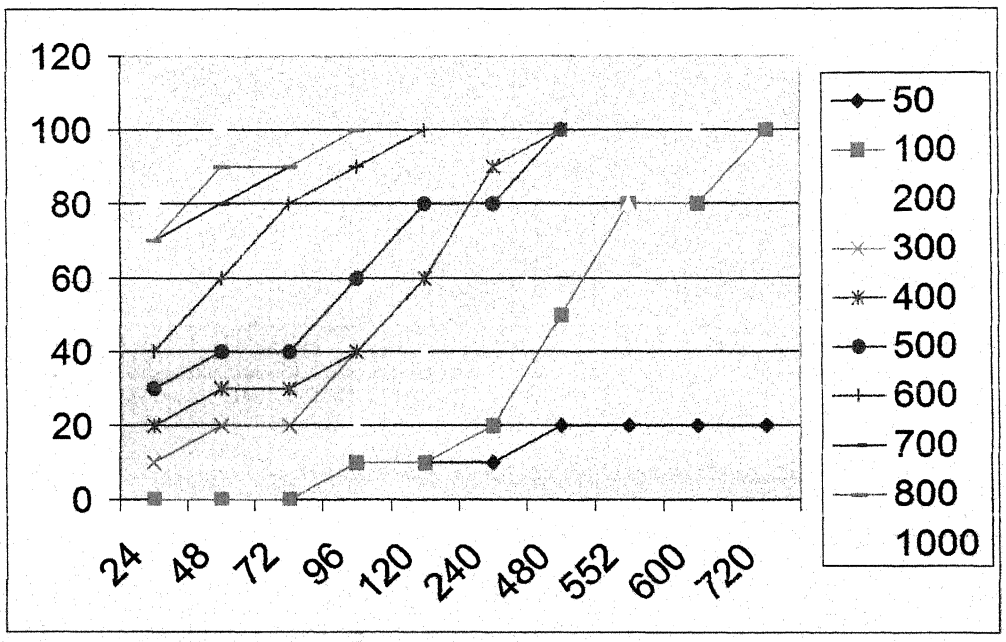
96 hrs. Median lethal concentration determination of nickel sulphate to *Clarias batrachus* by Probit Analysis

Figure –19



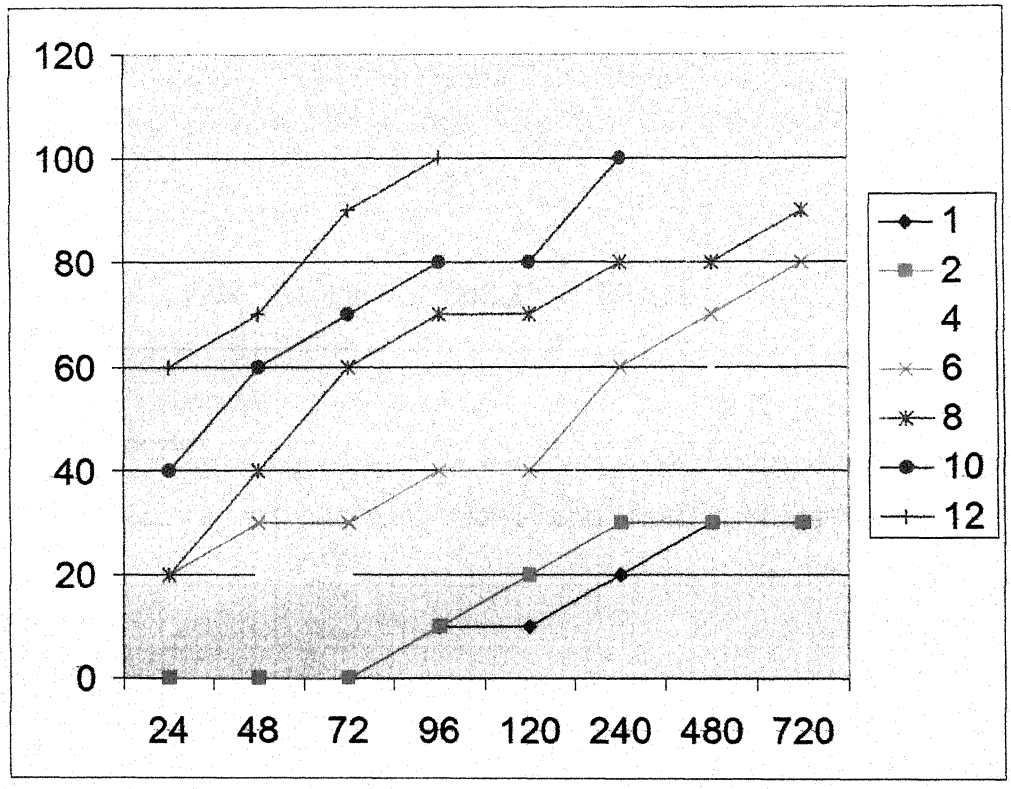
Effect of exposure duration on mortality of *Clarias batrachus* in different concentration of Cadmium Chloride

Figure -20



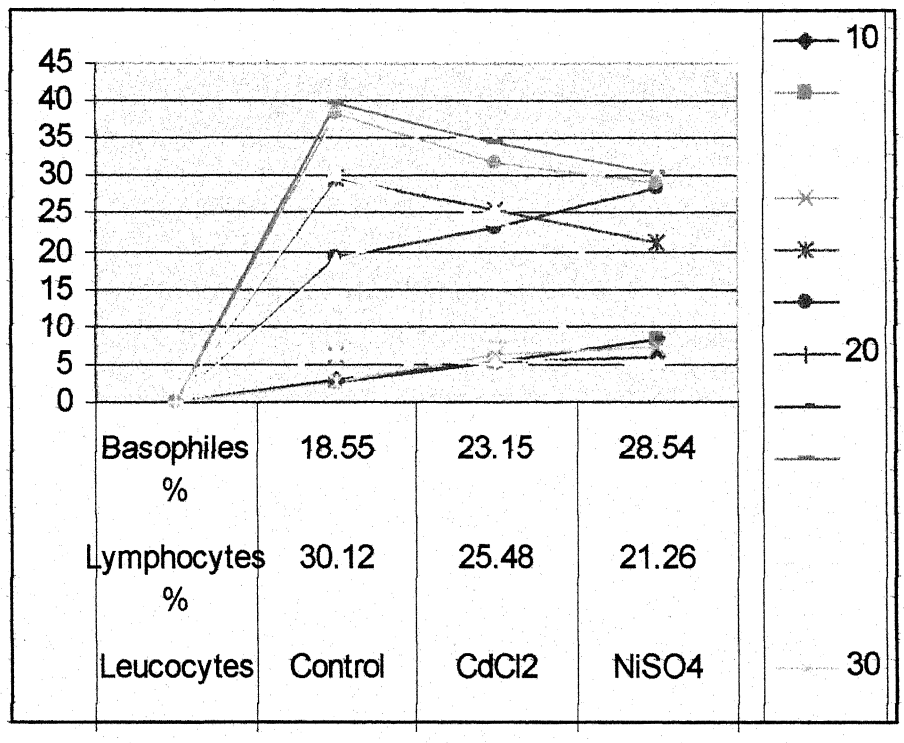
Effect of exposure duration on mortality of *Labeo rohita* in different concentration of mercuric chloride

Figure –21



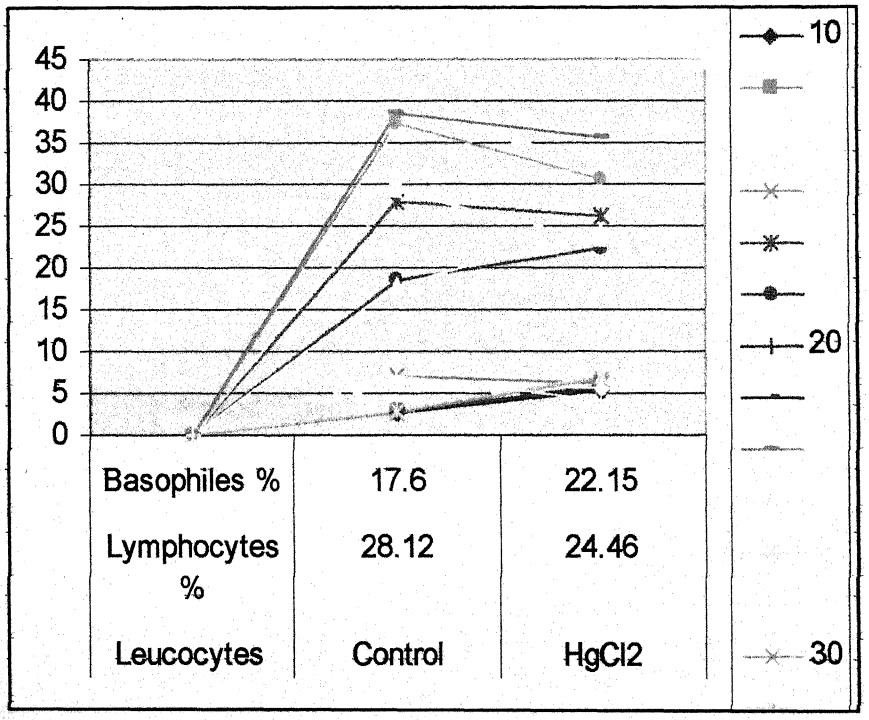
Effect of exposure duration on mortality of *Clarias batrachus* in different concentration of nickel sulphate

Figure-22



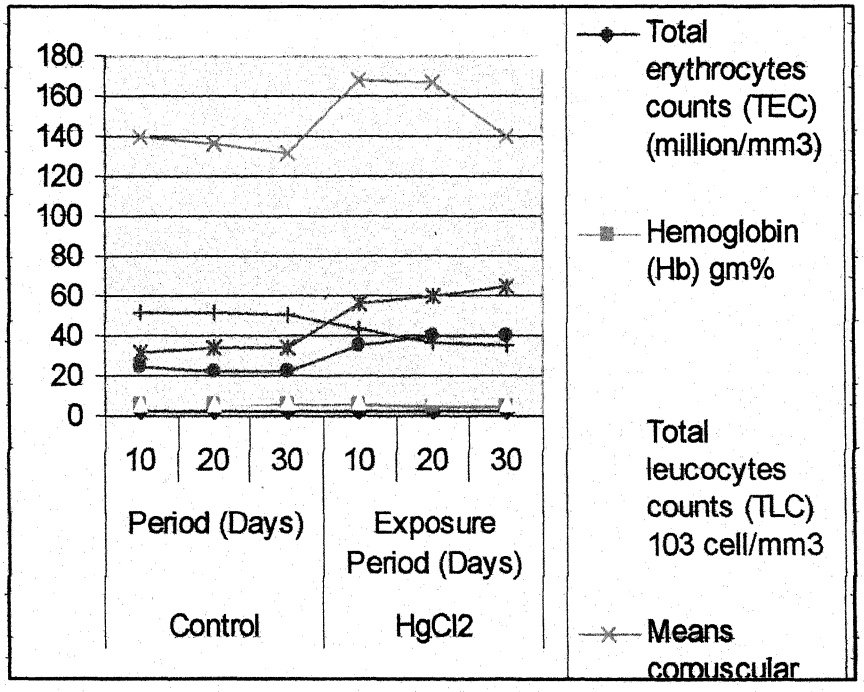
Difference Leucocytes count (DLC) of *Clarias batrachus* of sublethal concentration of heavy metals cadmium chloride and Nickel sulphate

Figure-23



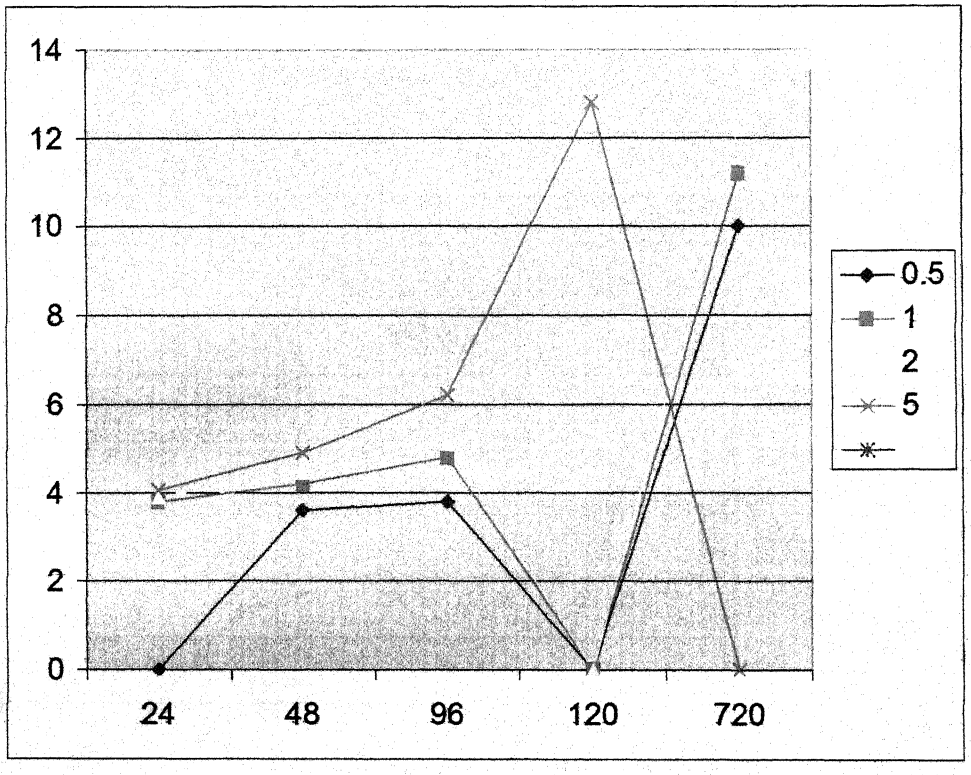
Differential Leucocytes count (DLC) of *Labeo rohita* of sublethal concentration of heavy metals mercuric chloride.

Figure-24



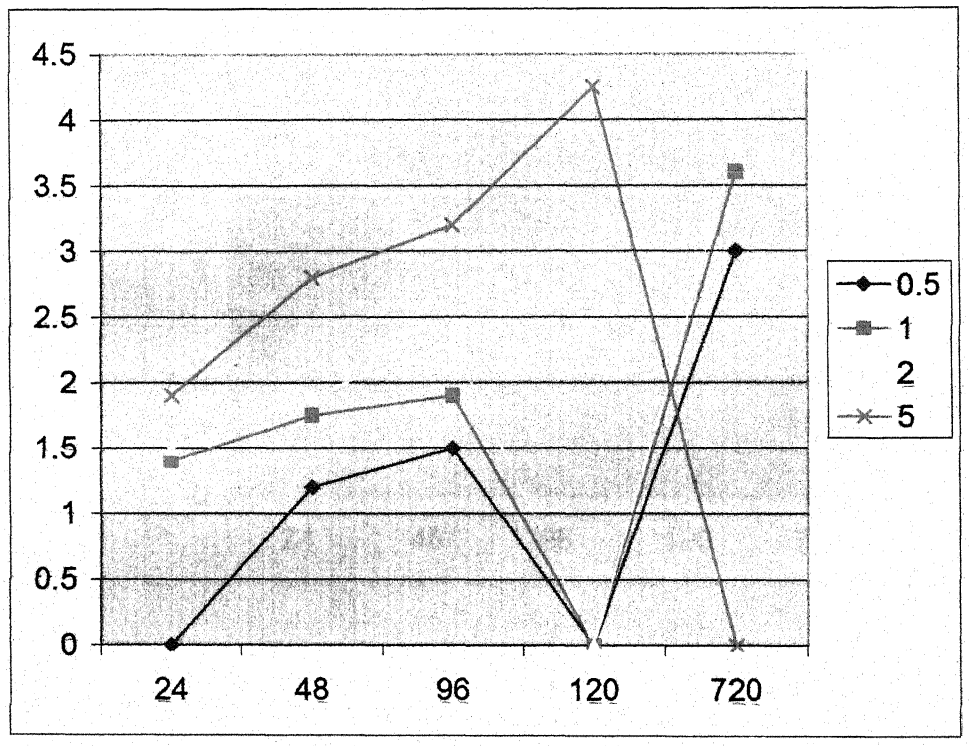
Alternation in total erythrocytes count (TEC), Hemoglobin (Hb), Total leucocytes counts (TLC), Means corpuscular Hemoglobin concentration (MCHC), Packed cell volume (PVC), following exposure to subleathal concentration mercuric chloride.

Figure –25



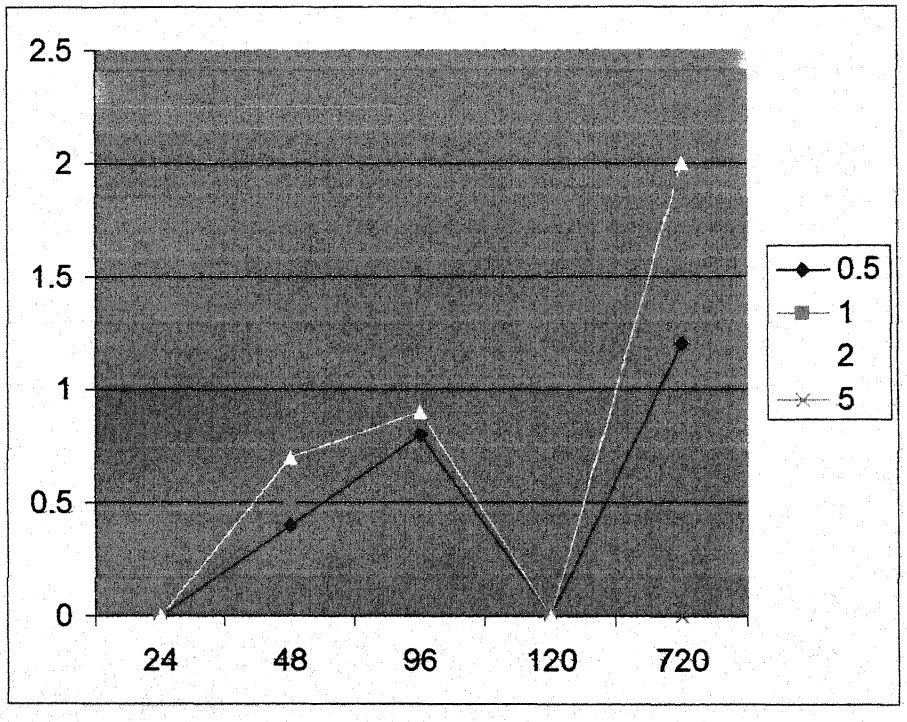
Accumulation of cadmium in gill of *Clarias batrachus* in different exposure time in different concentration of cadmium chloride

Figure –26



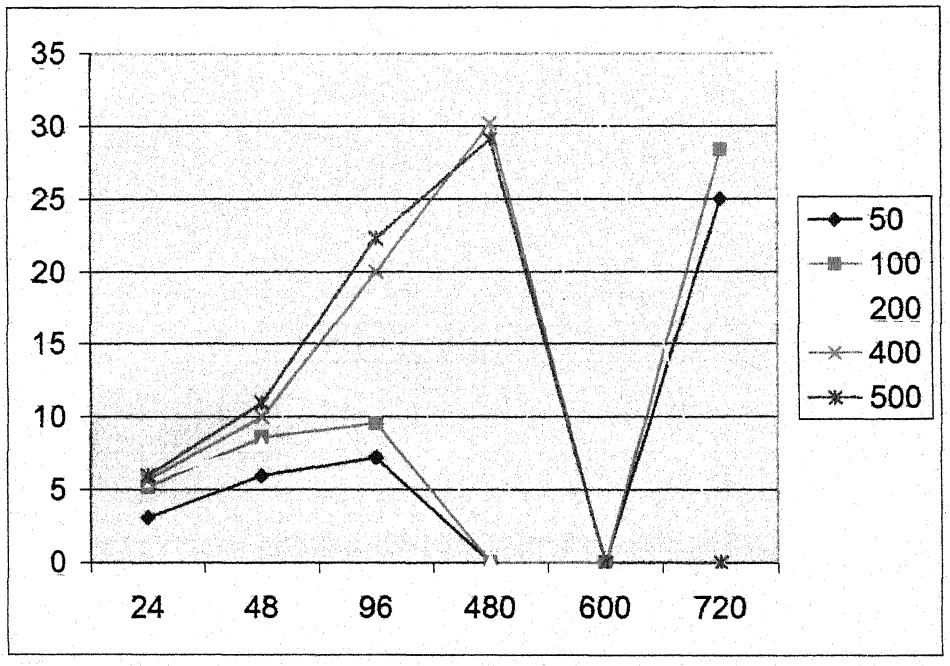
Accumulation of cadmium in liver of *Clarias batrachus* in different exposure time in different concentration of cadmium chloride

Figure –27



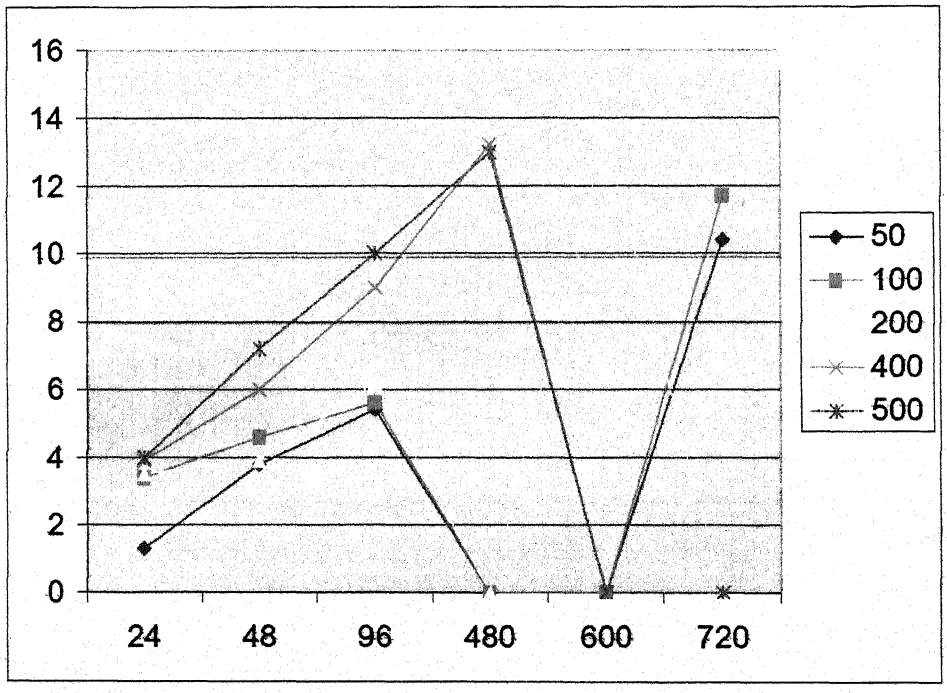
Accumulation of cadmium in kidney of *Clarias batrachus* in different exposure time in different concentration of cadmium chloride

Figure –28



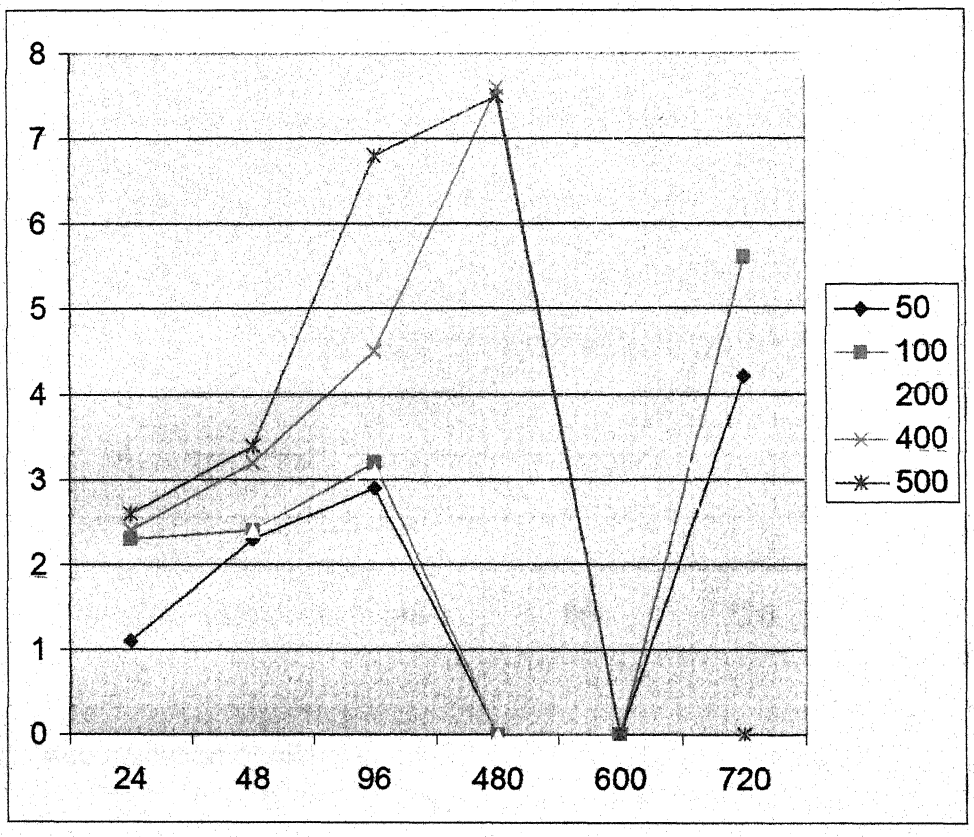
Accumulation of mercury in gills of *Labeo rohita* in different ` exposure time in different concentration of mercuric chloride

Figure –29



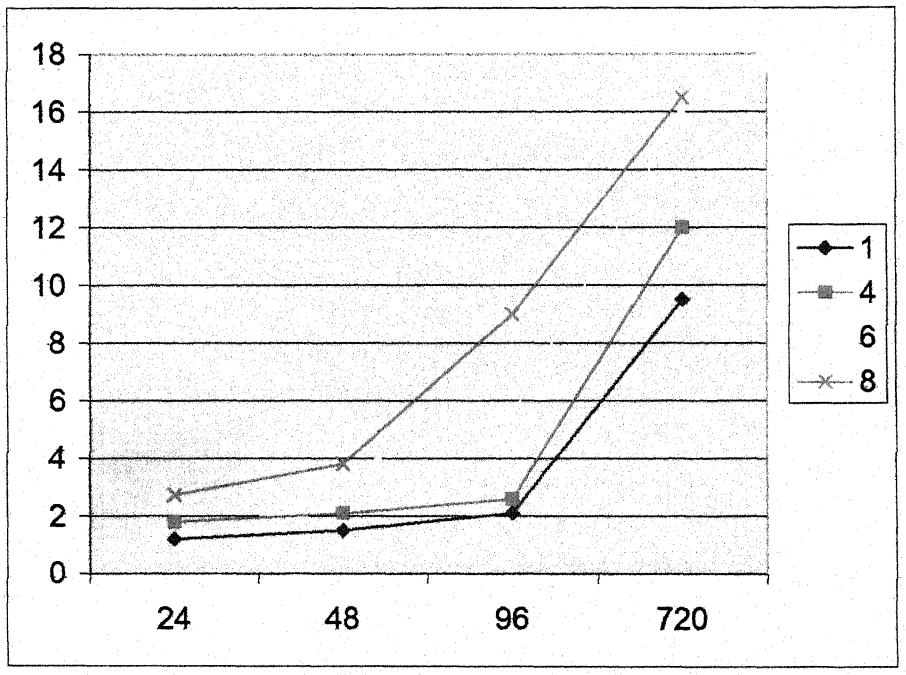
Accumulation of mercury in liver of *Labeo rohita* in different ` exposure time in different concentration of mercuric chloride

Figure –30



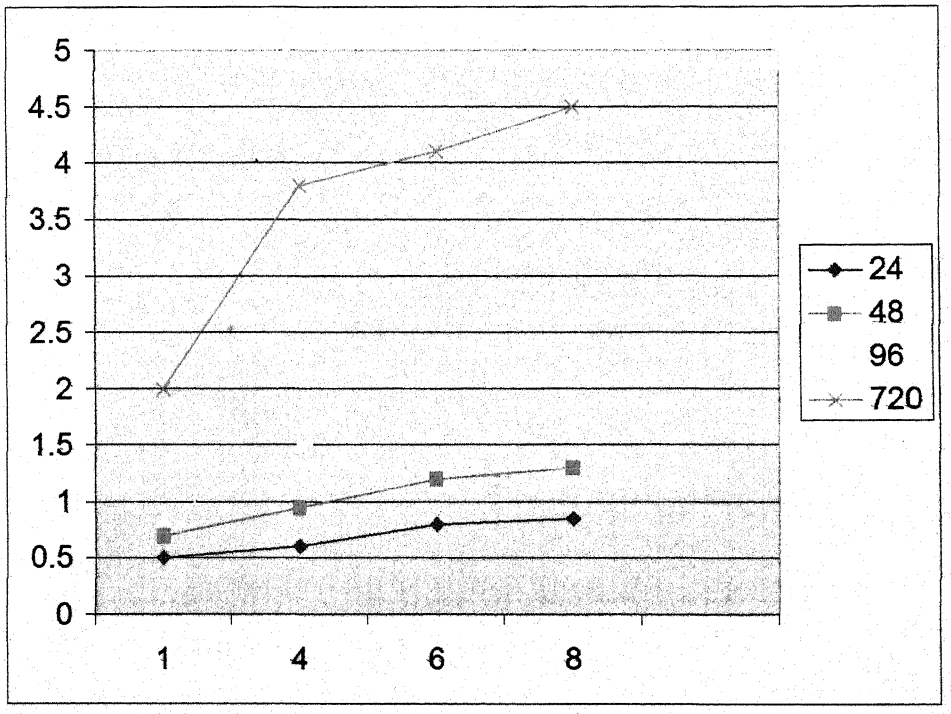
Accumulation of mercury in kidney of *Labeo rohita* in different exposure time in different concentration of mercuric chloride

Figure –31



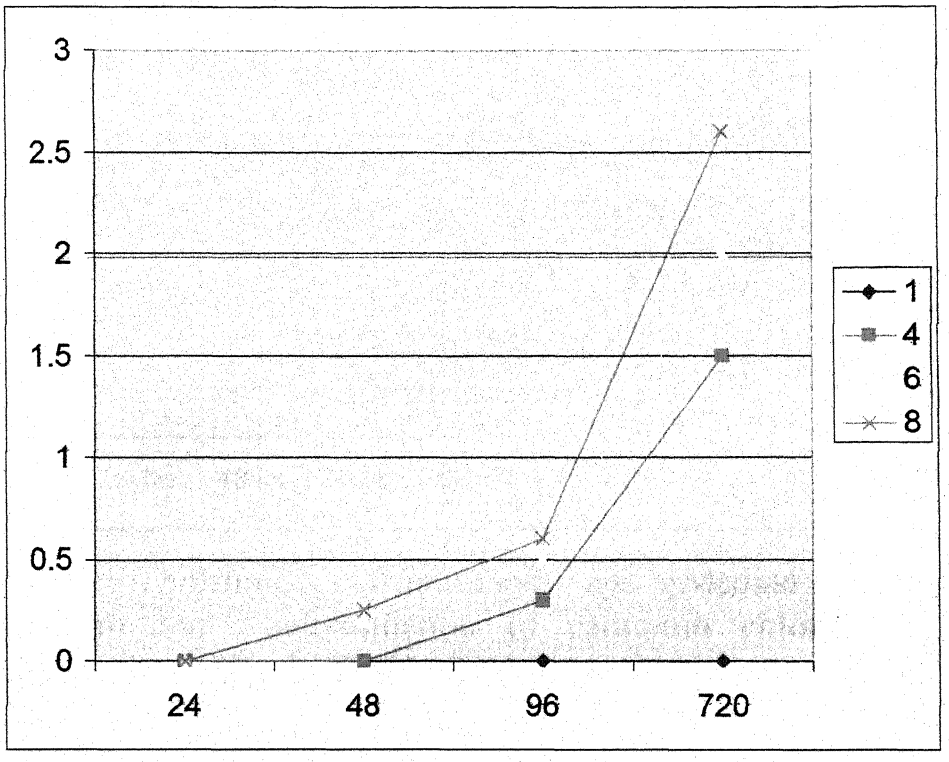
Accumulation of nickel in gills of *Clarias batrachus* in different ` exposure time in different concentration of nickel sulpahte

Figure –32



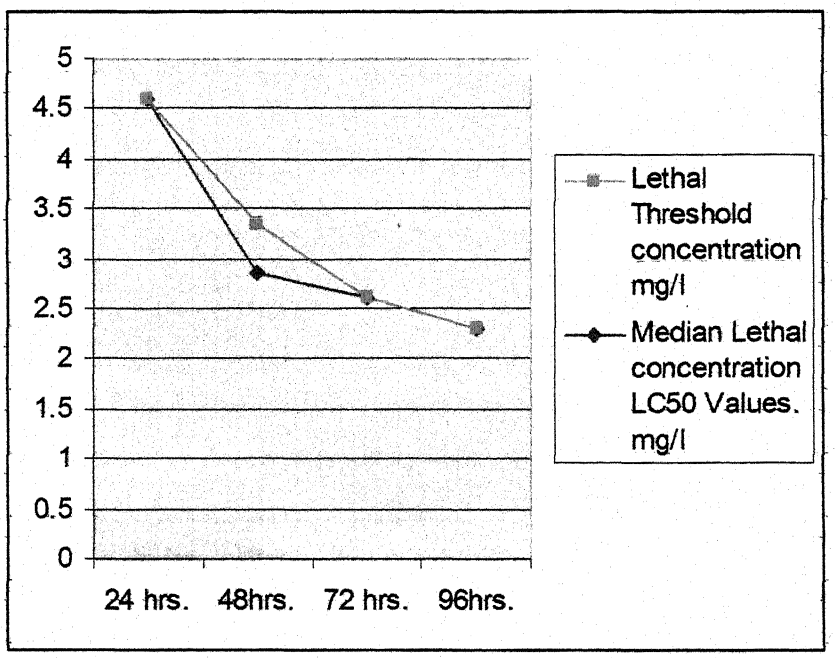
Accumulation of nickel in liver of *Clarias batrachus* in different ` exposure time in different concentration of nickel sulpahte

Figure –33



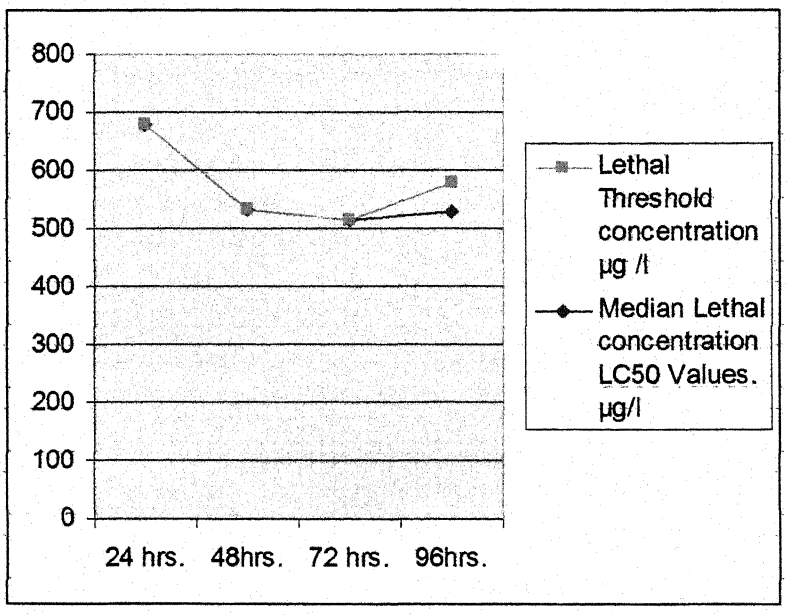
Accumulation of nickel in kidney of *Clarias batrachus* in different exposure time in different concentration of nickel sulpahte

Figure-34



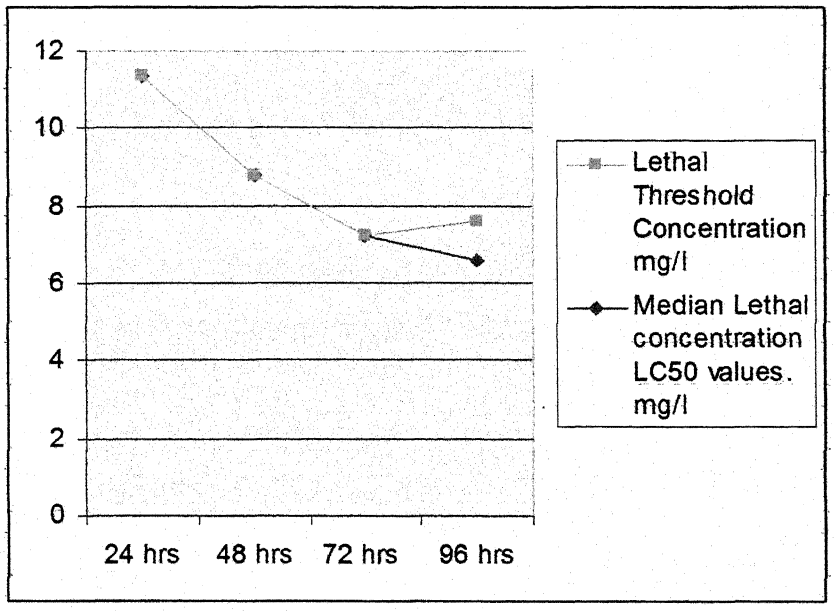
Lethal Threshold concentration and Median lethal concentration determination of cadmium chloride to *Clarias batrachus* by Probit Analysis

Figure-35

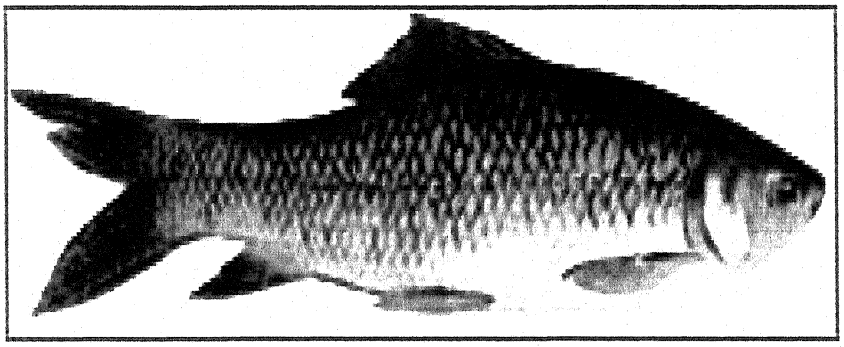


Lethal Threshold concentration and Median lethal concentration determination of mercuric chloride to *Labeo rohita* by Probit Analysis

Figure-36

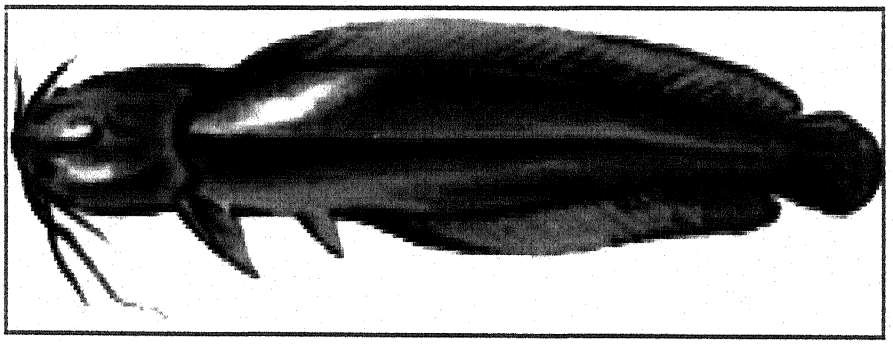


Lethal Threshold concentration and Median lethal concentration determination of nickel sulphate to Clarias batrachus by Probit Analysis



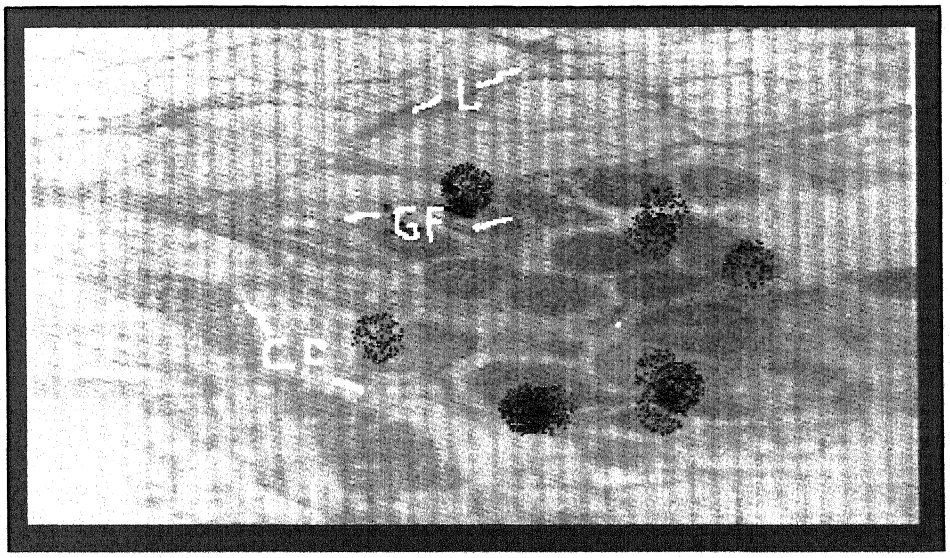
Labeo rohita

Family	:	Cyprinidae
Species	:	*Labeo rohita*
English name	:	Rohu, Indian major carp
Local name	:	Rohu
Maximum length	:	upto 94 cm
Habitat	:	Pond/River.
Availability	:	Good

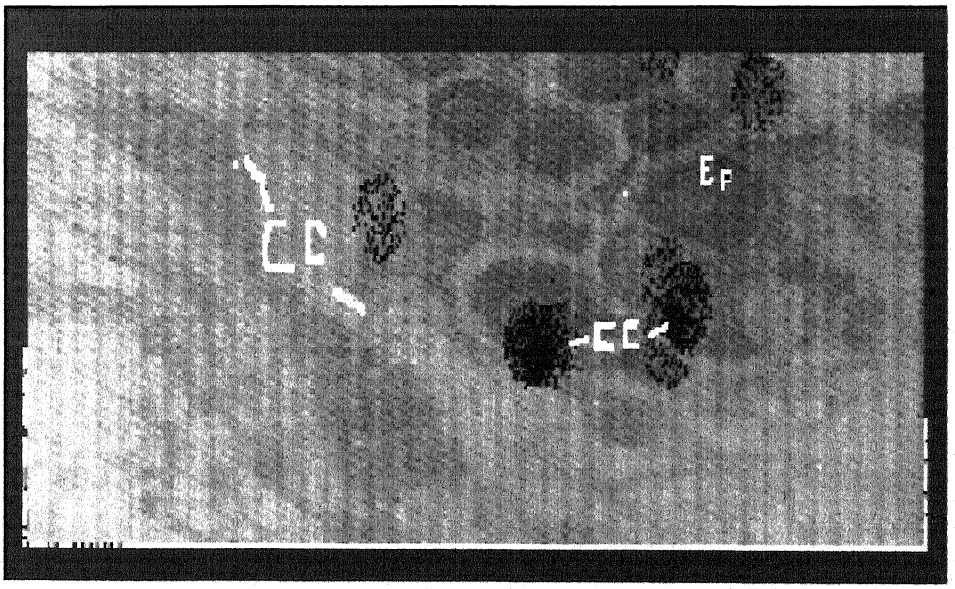


Clarias batrachus

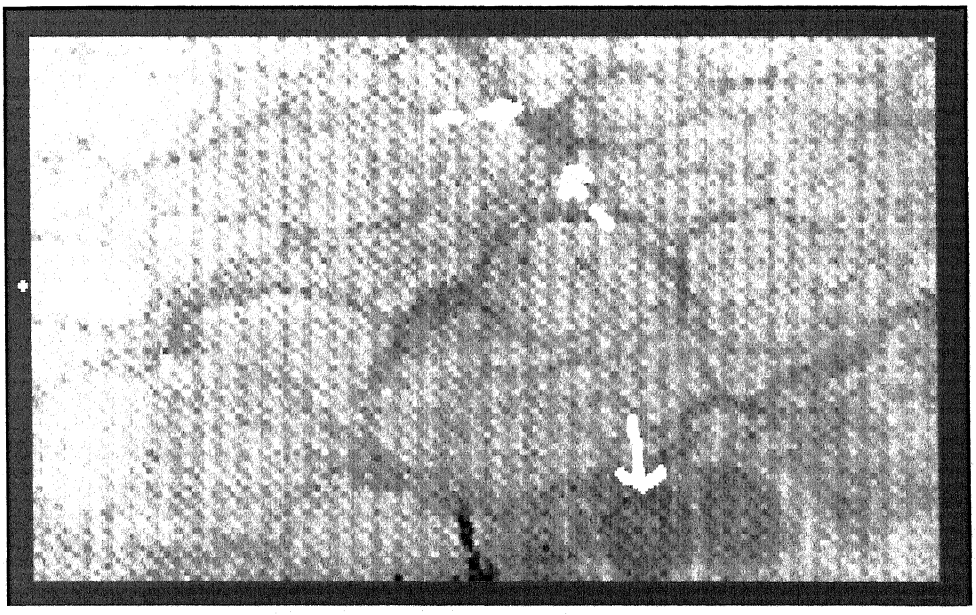
Family	:	Clariidae
Species	:	*Clarias batrachus*
English name	:	Stinging Cat fish
Local name	:	Magur
Maximum length	:	upto 30 cm
Habitat	:	Pond/Swamps
Availability	:	Fair.
	:	



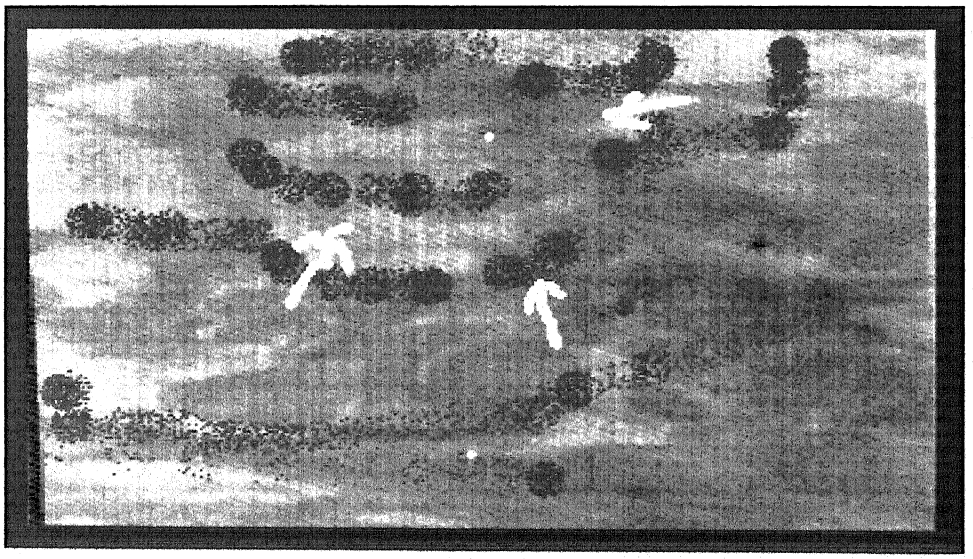
P1 V.S. of gill of controls *Clarias batrachus* showing primary gill lamella. Gill filament (Gf), Secondary Gill lamella (L), central core (CC) H&E x 100



P2 V.S. of gill of controls *Clarias batrachus* magnified, Gill filament, Pilaster cell (Pc), Epithelial cell (Ep), central core (CC) 400

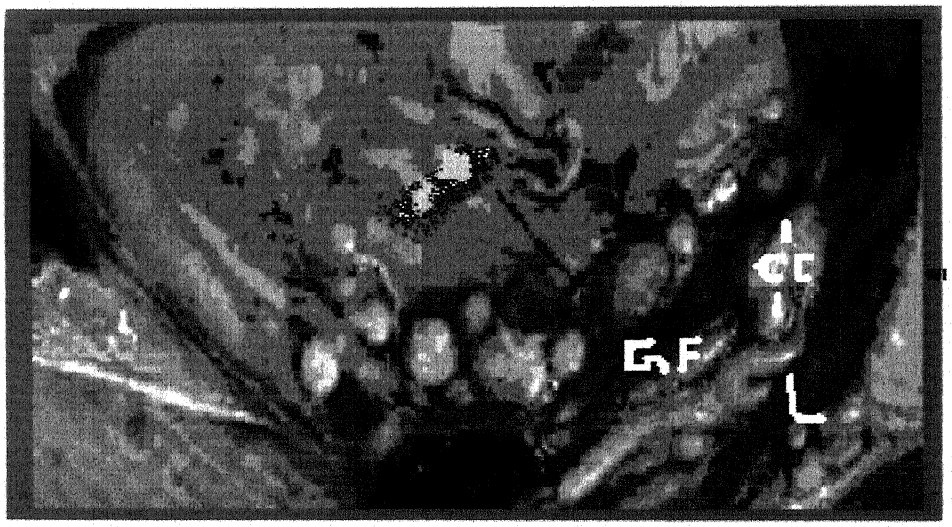


P3 V.S. of gill of controls *Clarias batrachus* after 24 hrs. exposure to 1.0 mg/l cadmium chloride depicting fusion of secondary gill lamella (broken arrow) and degeneration of epithelial cell (arrow) H&E x 400

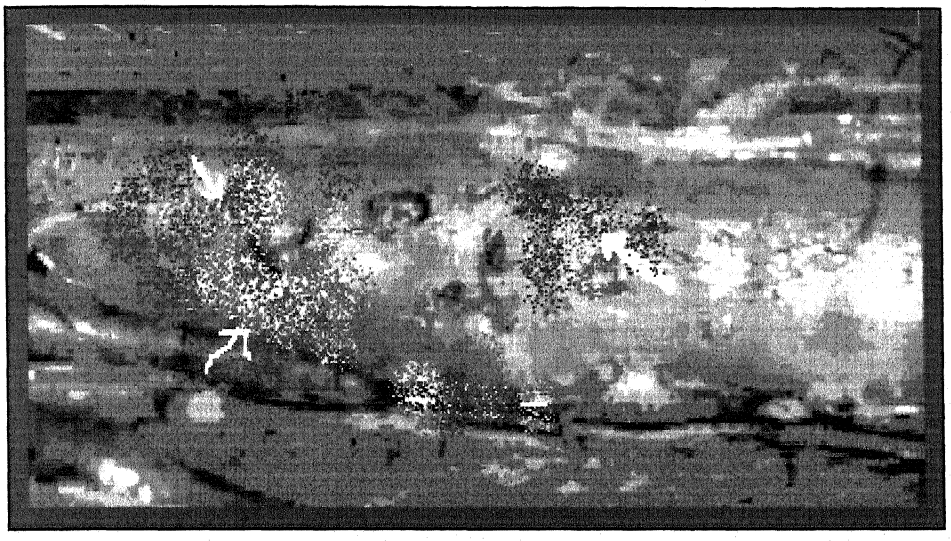


P4 V.S. of gill of *Clarias batrachus* after 10days exposure to 2.0 mg/l cadmium chloride depicting swelling at the tips of secondarlamella (arrow) H&E x 400

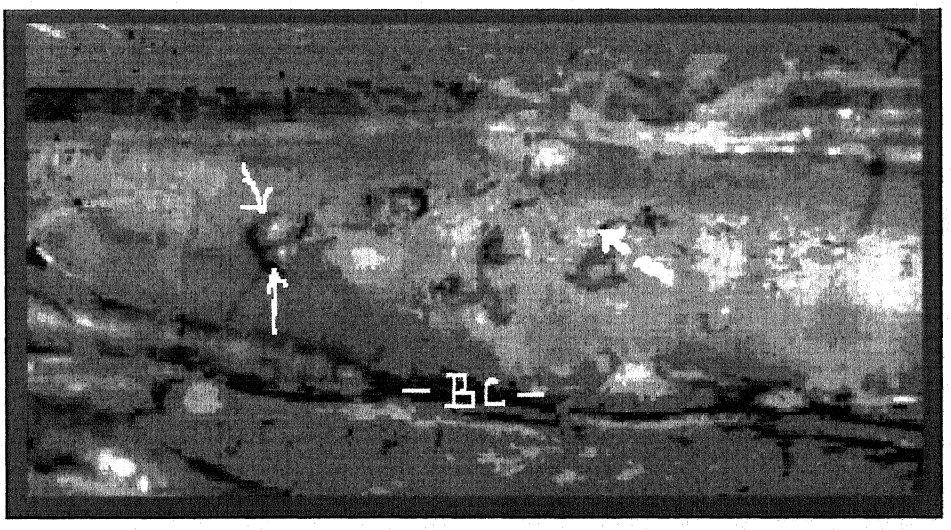
P5 V.S. of gill of *Clarias batrachus* after 20days. exposure to 2.0 mg/l cadmium chloride depicting necrosis changes in epithelial cell (arrow) H&E x 400



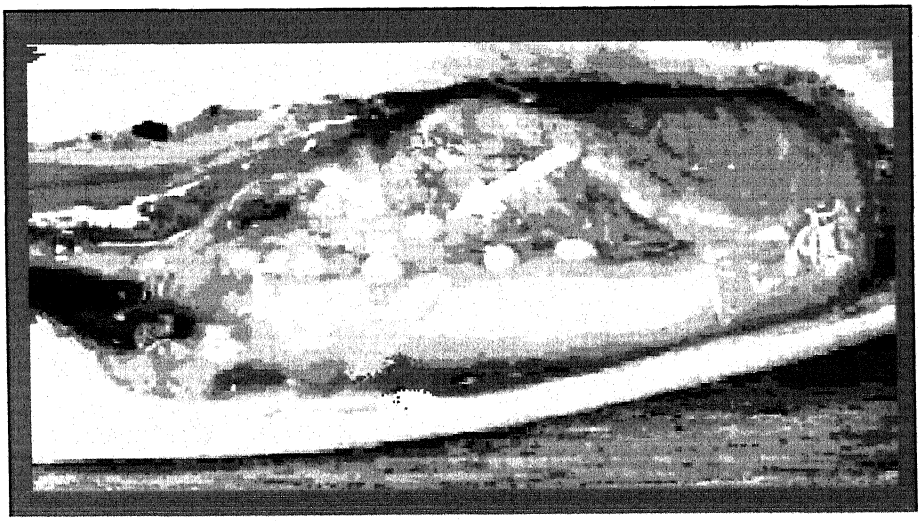
P6 V.S. of gill of control *Labeo rohita* showing Primary Gill lamella (Gill filament, (Gf), Secondary gill lamella (L), Central core (CC) H&E x 400



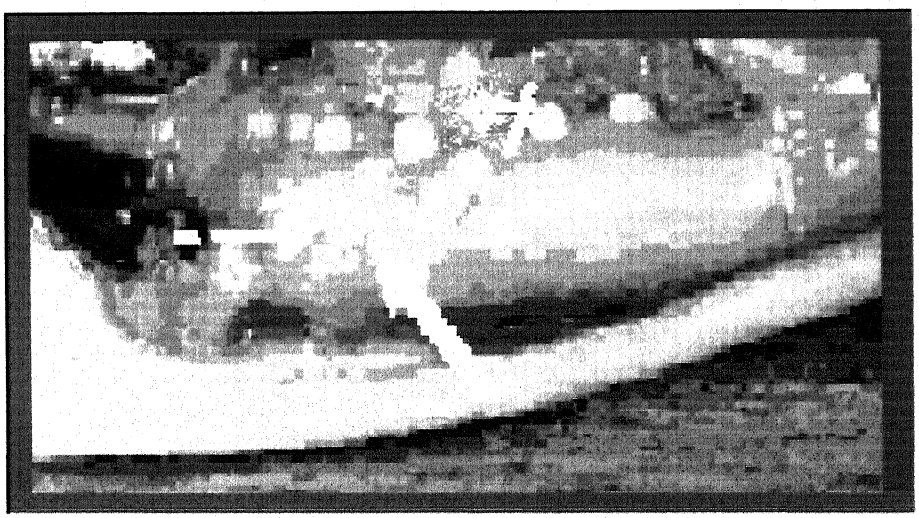
P7 V.S. of gill of Labeo *rohita* after 48 hrs. exposure to 100 µg /l mercuric chloride showing excessive mucous secretion (arrow)H&E x 100



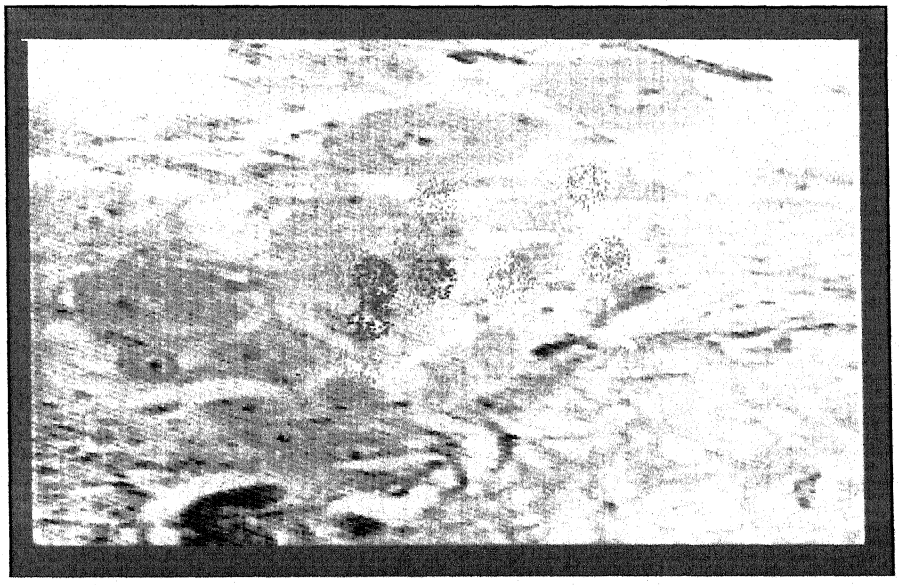
P8 V.S. of gill Labeo *rohita* after 96 hrs. exposure to 100 µg /l showing hyperaemia (arrow) and partial separation of epithelia cells (broken arrow), Blood capillary (BC) H&E x 400



P9 V.S. of gill of *Labeo rohita* after 10 days exposure to 200 µg/l mercuric chloride showing hyperaemia, fusion of secondary gill lamella (arrow) and necrosis in epithelial cell. H&E x 100

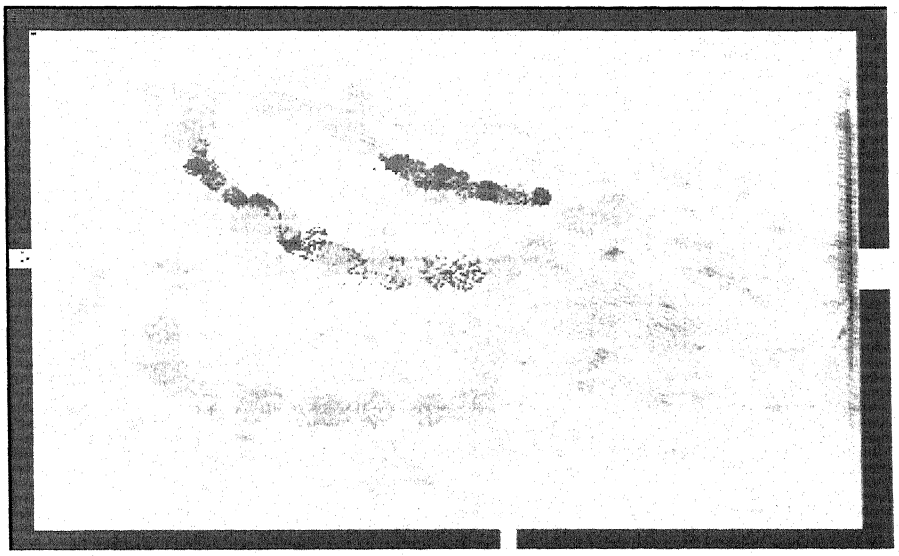


P10 V.S. of gill of *Labeo rohita* after 10 days exposure to 500 µg /l mercuric chloride showing hyperaemia,(broken arrow),degeneration of epithelial cell (arrow) and hypertrophy (x) . H&E x 400

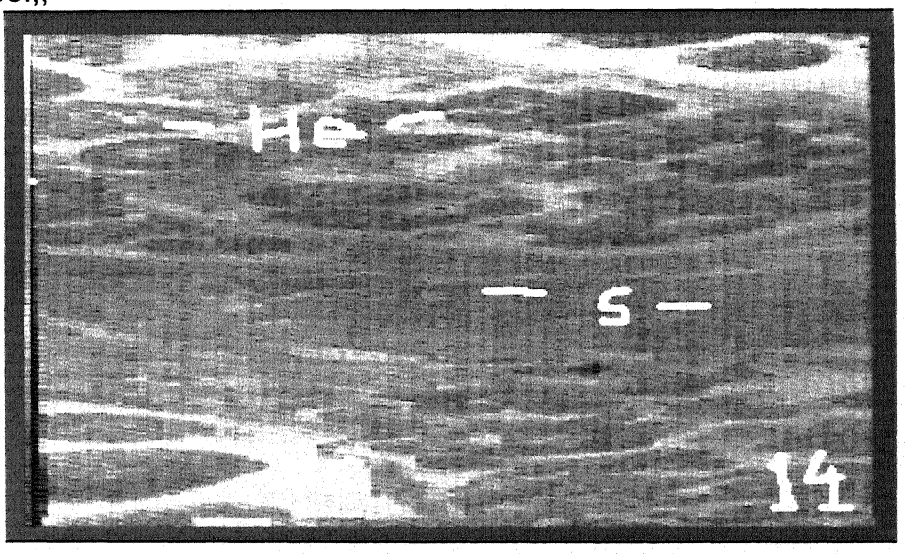


P11 V.S. of gill of *Clarias batrachus* after 24 hrs exposure to 2.0 mg/l nickel sulphate showing excessive secretion of mucous (broken arrow) H&E x 400

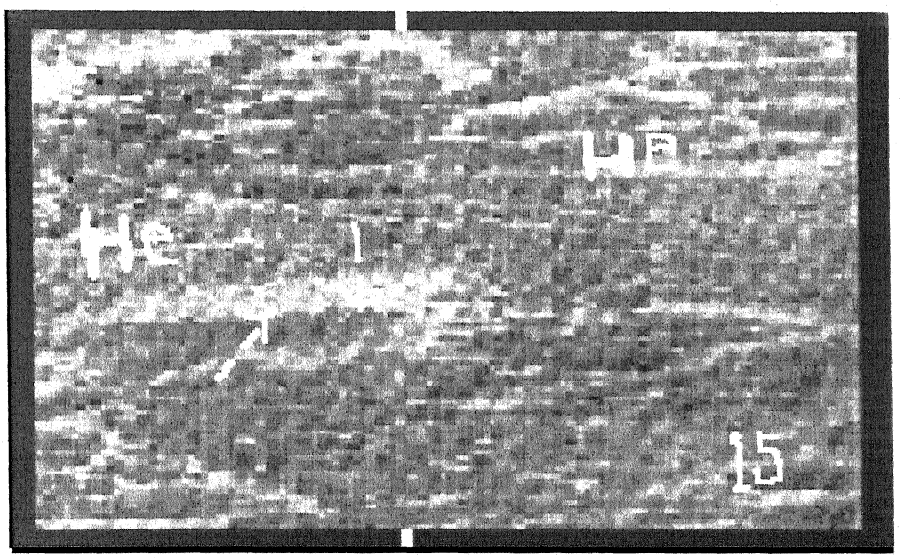
P12 V.S. of gill of *Clarias batrachus* after 10 days exposure to 6.0 mg/l nickel sulphate showing partial separation of the epithelial cells from basement membrane (arrow) H&E x 40



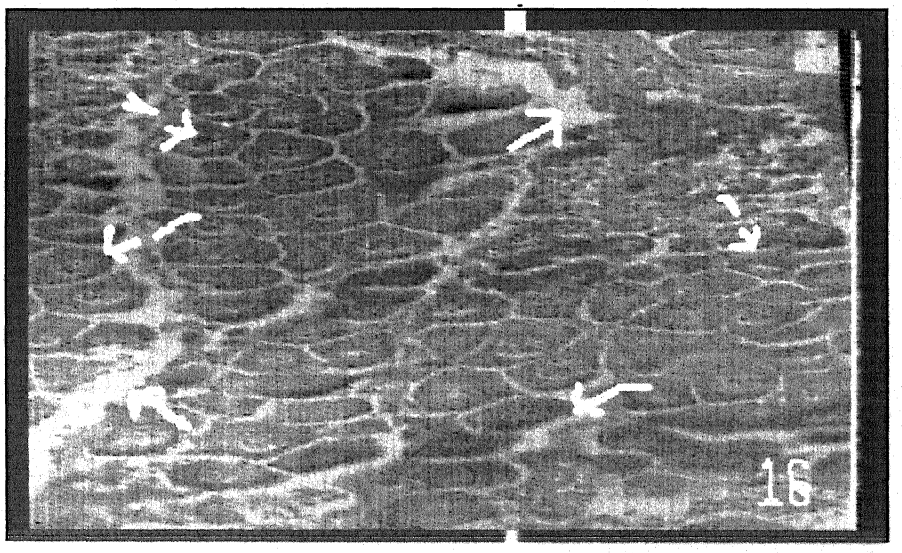
P13 V.S. of gill of *Clarias batrachus* after 20 days exposure to 6.0 mg/l nickel sulphate showing swollen epithelial lining (arrow) H&E x 400.;;



P14 V.S. of liver of control *Clarias batrachus* showing mesh like arrangement of hepatocytes (He), sinusoids (S), Blood capillary (Bc) H&E x 100



P15 V.S. of liver of *Clarias batrachus* after 5 days exposure to 2.0 mg/l cadmium chloride showing vacuolar degeneration of hepatocytes (He) (arrow) and hyperaemia (broken arrow) H&E x 400



P16 V.S. of liver of *Clarias batrachus* after 20 days exposure to 3.0 mg/l cadmium chloride showing vacuolar degeneration of cytoplasm, eccent displacement of nuclei (broken arrow) and profuse haemorrhage (arrow) H&E x 400

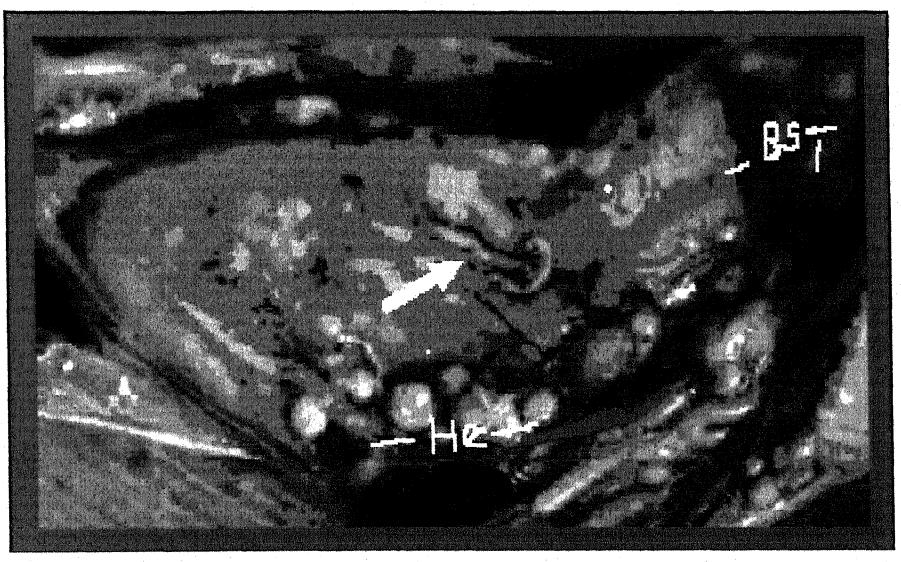
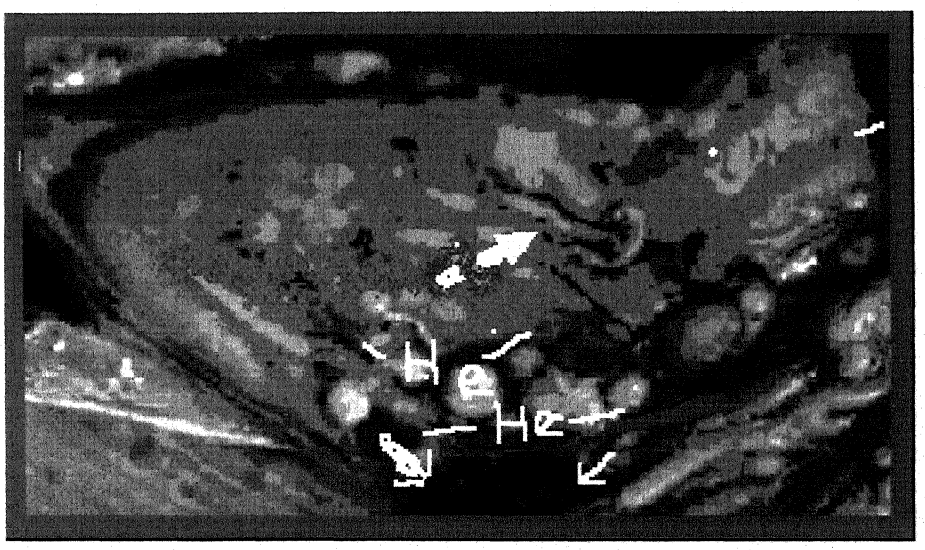
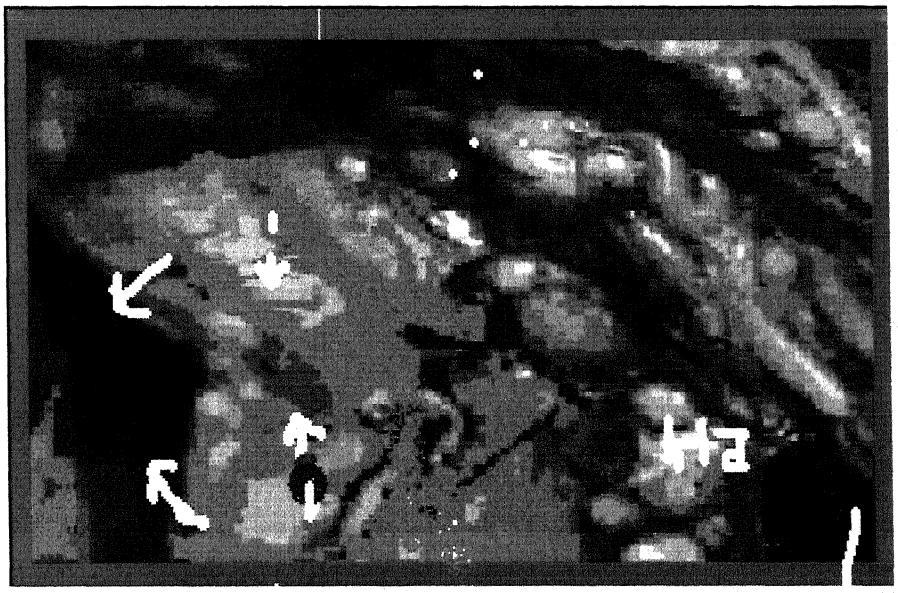


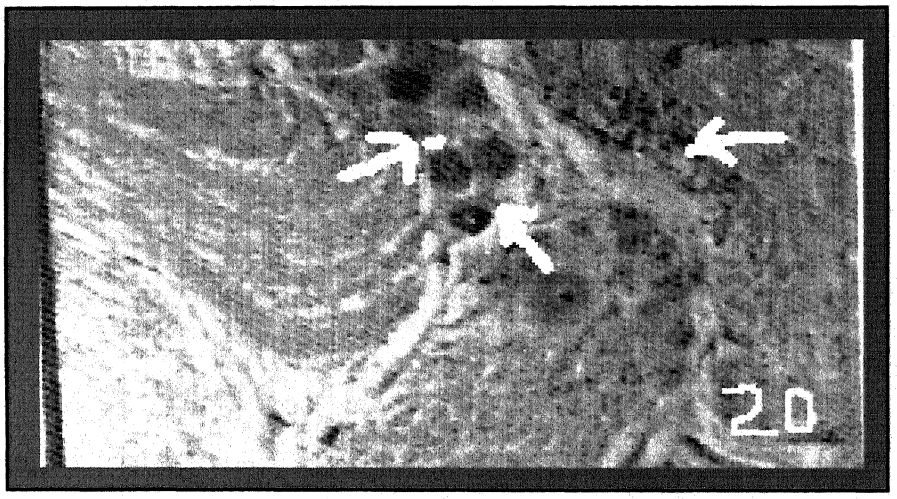
Fig 17 Liver tissue in Indian carp *Labeo rohita* after 20 days exposure to 50.0 µg /l mercuric chloride showing vacuolar degeneration of hepatocytes (arrow) and Blood space (BS) H&E x 400 .



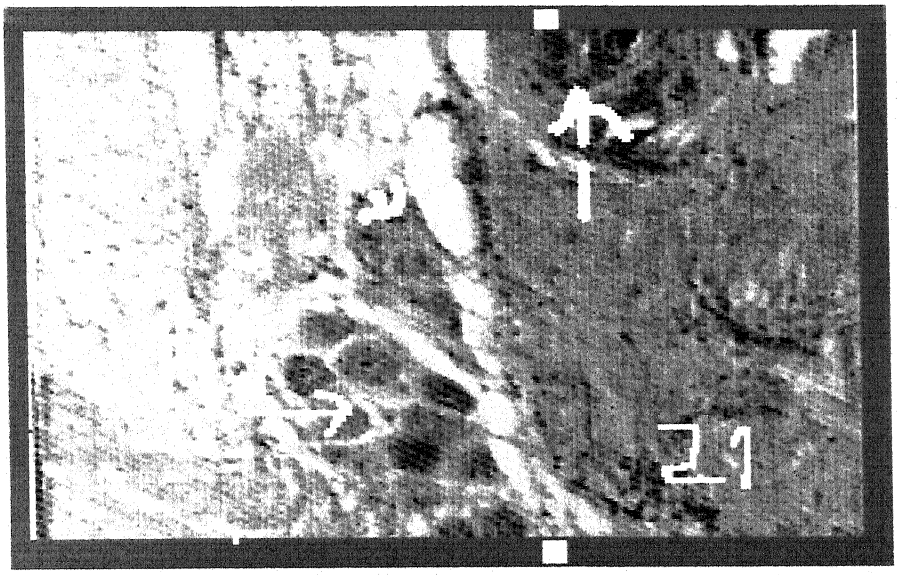
P18 V.S. of liver of *Labeo rohita* after 5 days exposure to 200.0 µg/l mercuric chloride showing vacuolar degeneration of hepatocytes (broken arrow)eccentric of nuclei (arrow) H&E x 400



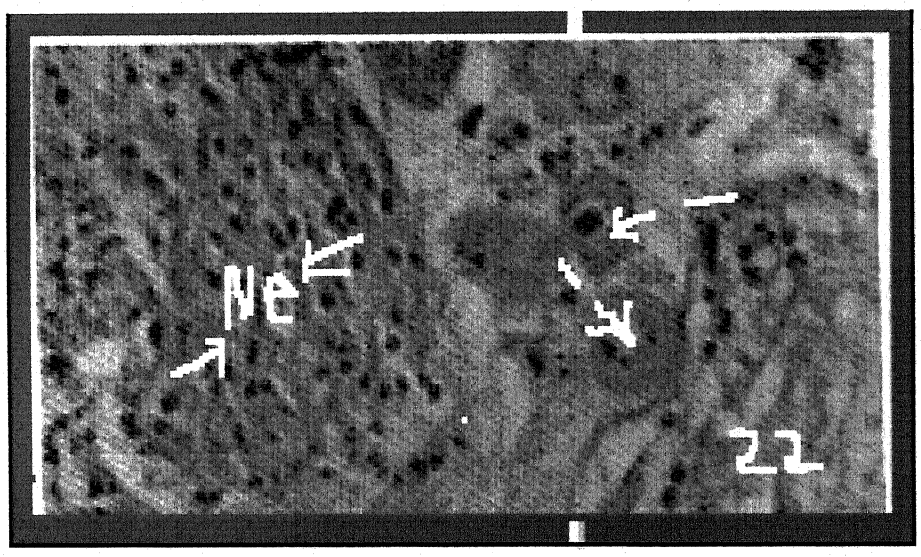
P19 V.S. of liver of *Labeo rohita* after 96 hrs exposure to 600.0 µg/l mercuric chloride showing vacuolar degeneration of cytoplasm (arrow) and hyperameia (Ha) of Blood capillaries (broken arrow), H&E x 400



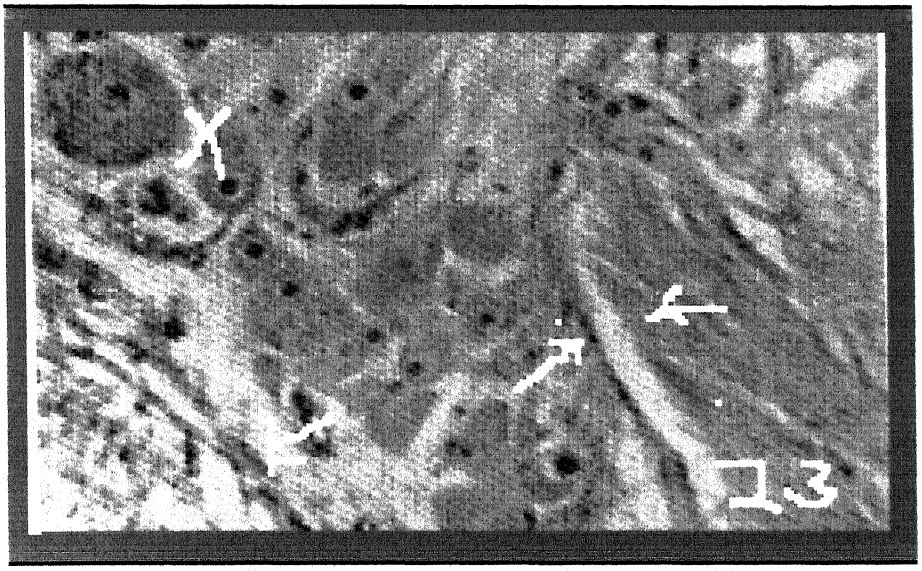
P20 V.S. of liver of *Clarias batrachus* after 20 days exposure



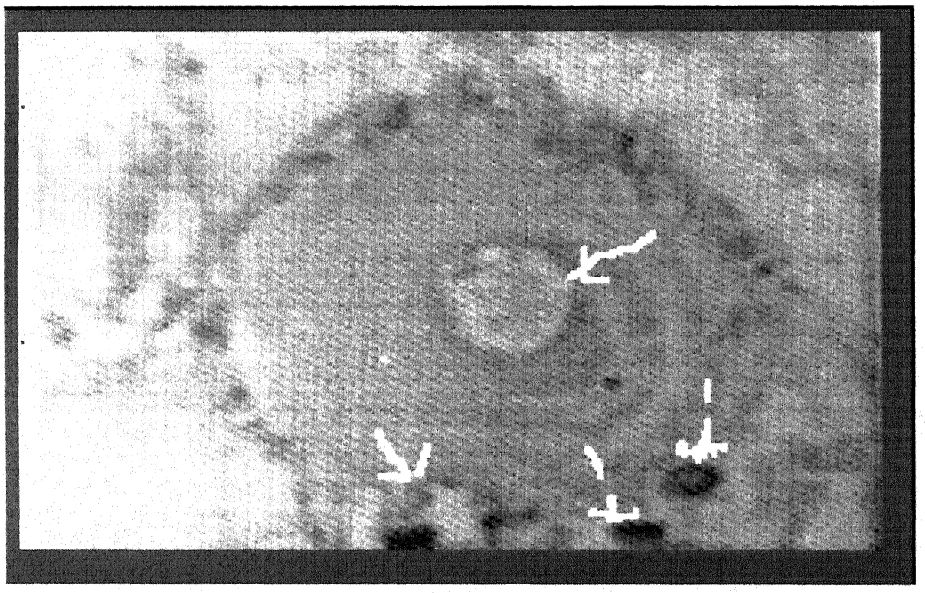
P21 V.S. of liver of *Clarias batrachus* after 10 days exposure to 4.0 mg/l nickel sulphate showing vacuolar degeneration (broken arrow), severe lesions and profuse haemorrhage (Ha) (arrow) H&E x 400



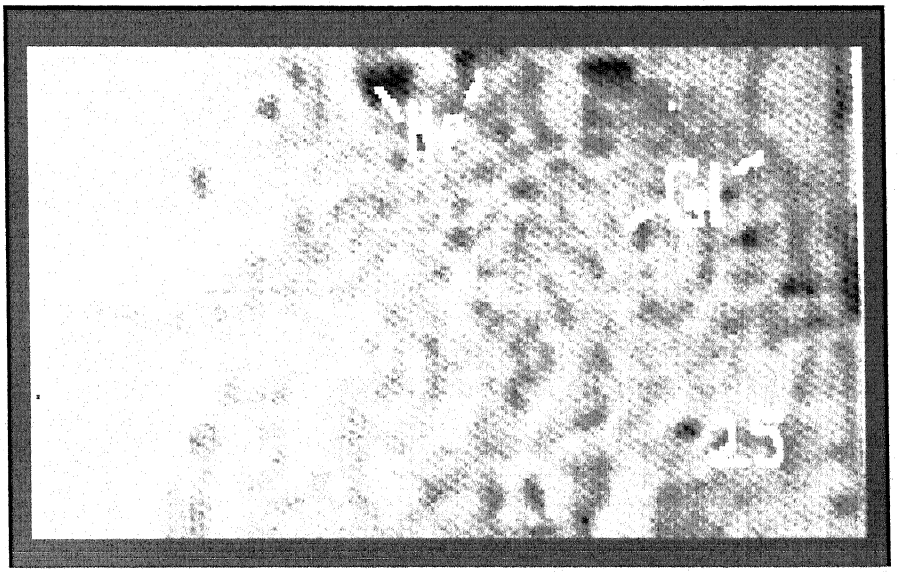
P22 V.S. of liver of *Clarias batrachus* after 20 days exposure to 6.0 mg/l nickel sulphate showing severe necrosis (Ne) (arrow) eccentric nuclei (broken arrow) and haemorrhage H&E x 400



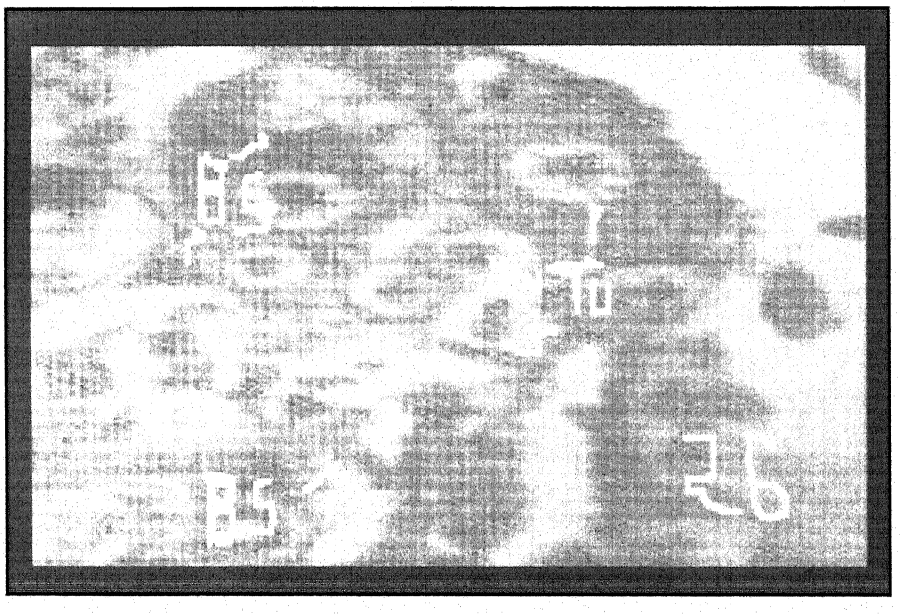
P23 V.S. of liver of *Clarias batrachus* after 10 days exposure to 8.0 mg/l nickel sulphate showing vacuolar degeneration (arrow), eccentric nuclei and haemorrhage (x) H&E x 400



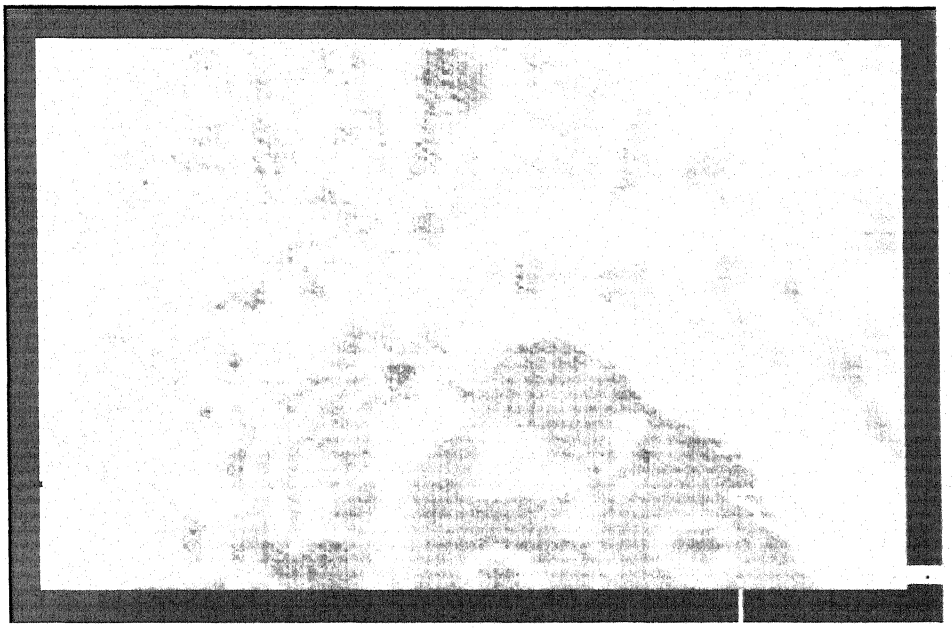
P24 V.S. of liver of *Clarias batrachus* after 10 days exposure to 10.0 mg/l nickel sulphate showing vacuolar degeneration (arrow) and severe haemorrhage (broken arrow) H&E x 400



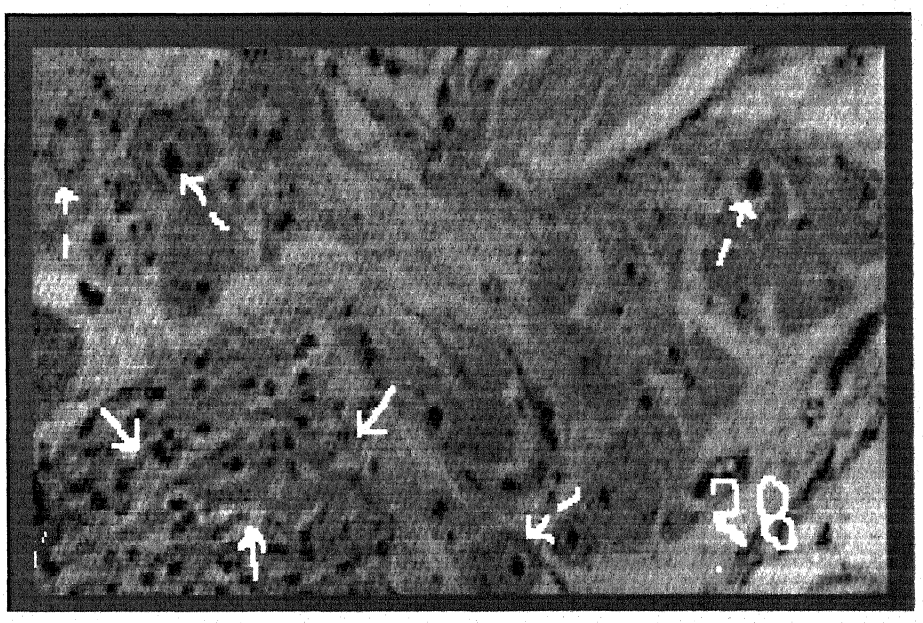
P25 T.S. of kidney of control *Clarias batrachus* showing normal structure of renal tubule (Glomerulus- Gl, Renal tubule-Rt, Haemopoietic tissue-HP),.



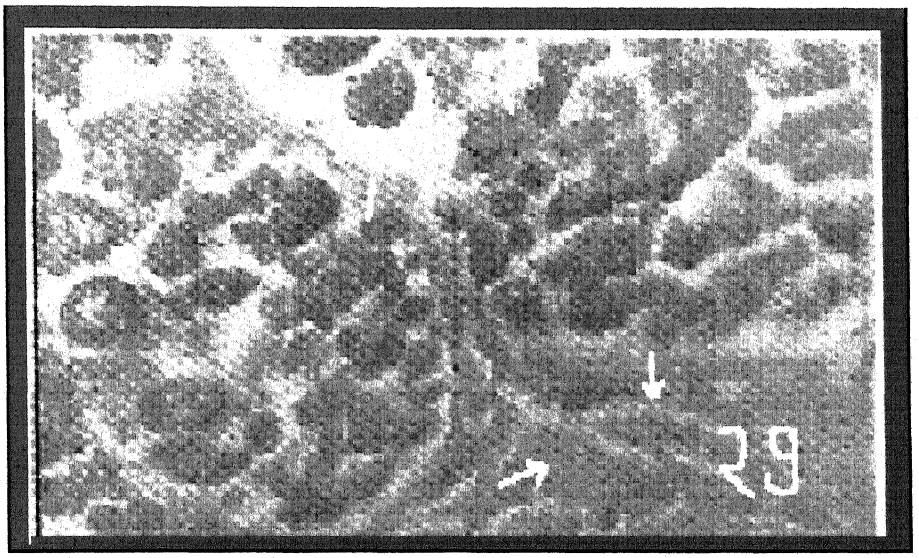
P26 T.S. of kidney of *Clarias batrachus* after 96 hrs exposure to 1.0 mg/l cadmium chloride showing hypertrophy (arrow) of epithelial cell of convoluted tubule (Tu) and Blood space (BS)H&E x 400



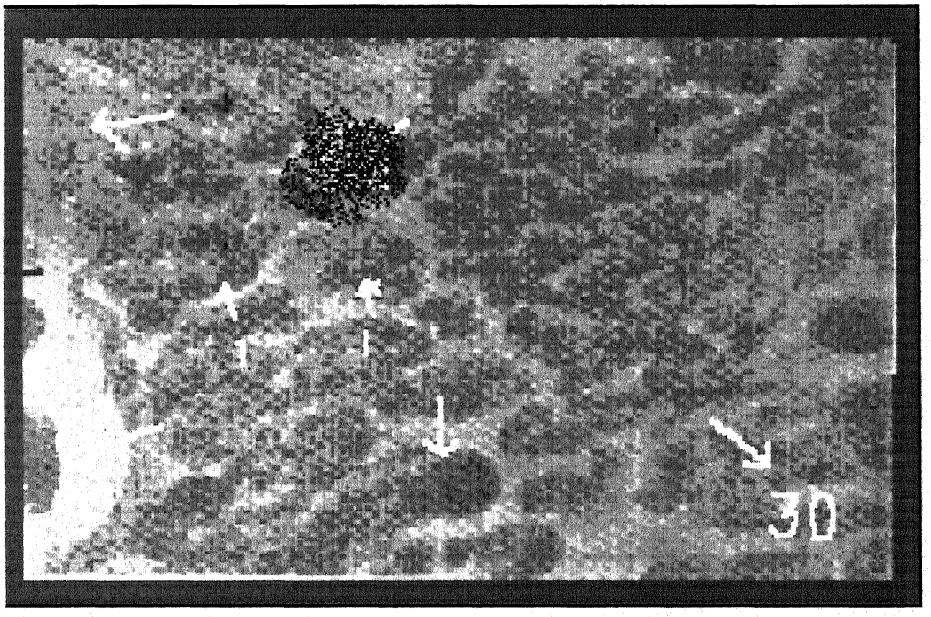
P27 T.S. of kidney of *Clarias batrachus* after 10 days exposure to 2.0 mg/l cadmium chloride showing haemorrhage (broken arrow) and necrosis (arrow), H&E x 400



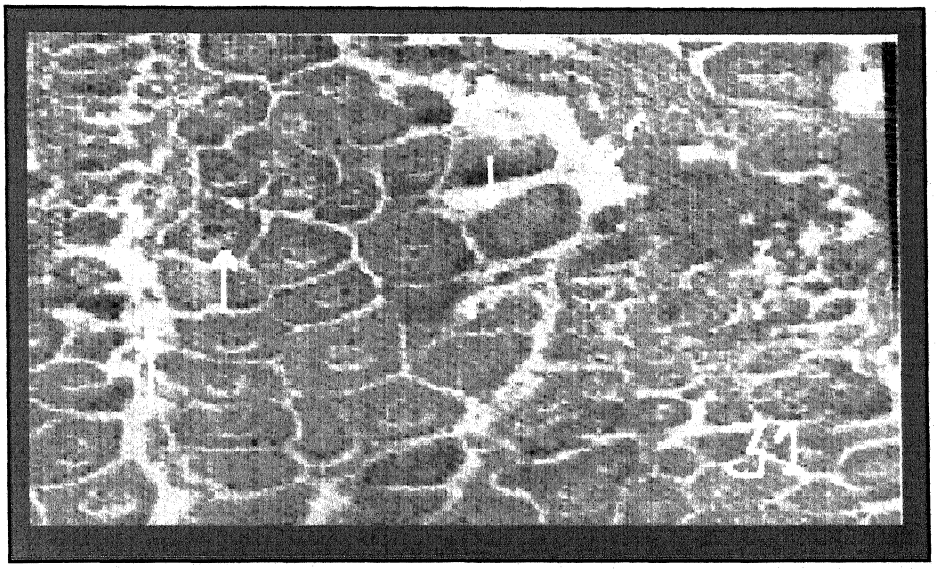
P28 T.S. of kidney of *Clarias batrachus* after 5 days exposure to 4.0 mg/l cadmium chloride showing degeneration of epithelial cell (broken arrow)and necrosis (arrow) H&E x 400
H&E x 400



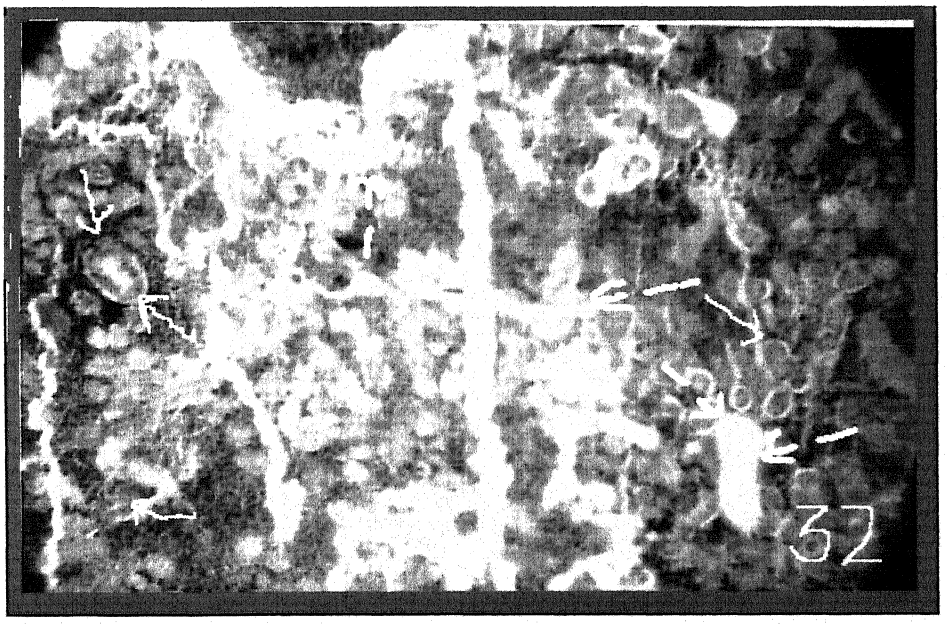
P29 T.S. of kidney of *Labeo rohita* after 48 hrs exposure to 100.0 µg/l mercuric chloride showing hypertrophy (broken arrow) of epithelial cell and necrosis (arrow) H&E x 400



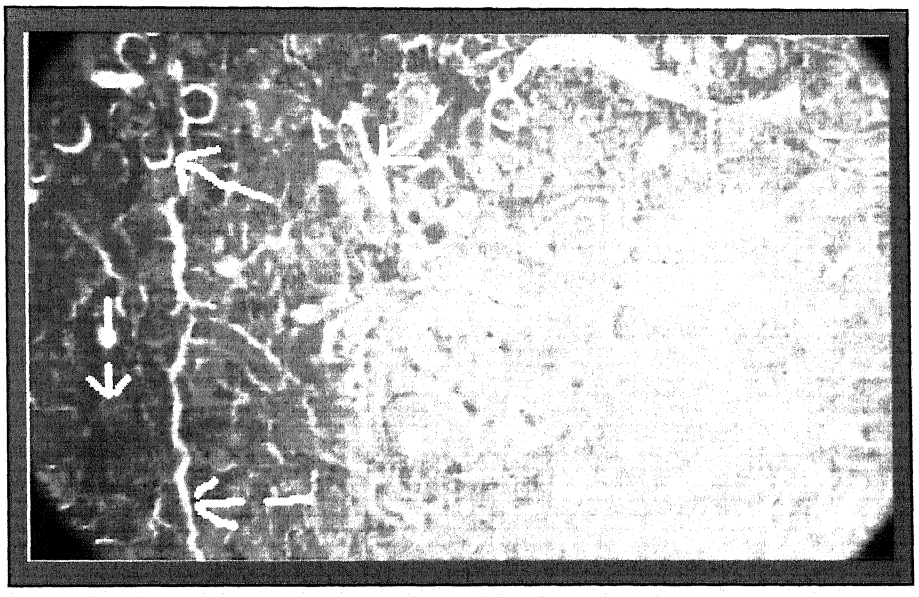
P30 T.S. of kidney of *Labeo rohita* after 96 hrs exposure to 200.0 µg /l mercuric chloride showing hyperplasia in epithelial cell (broken arrow), oedema and haemorrhage (arrow) H&E x 400



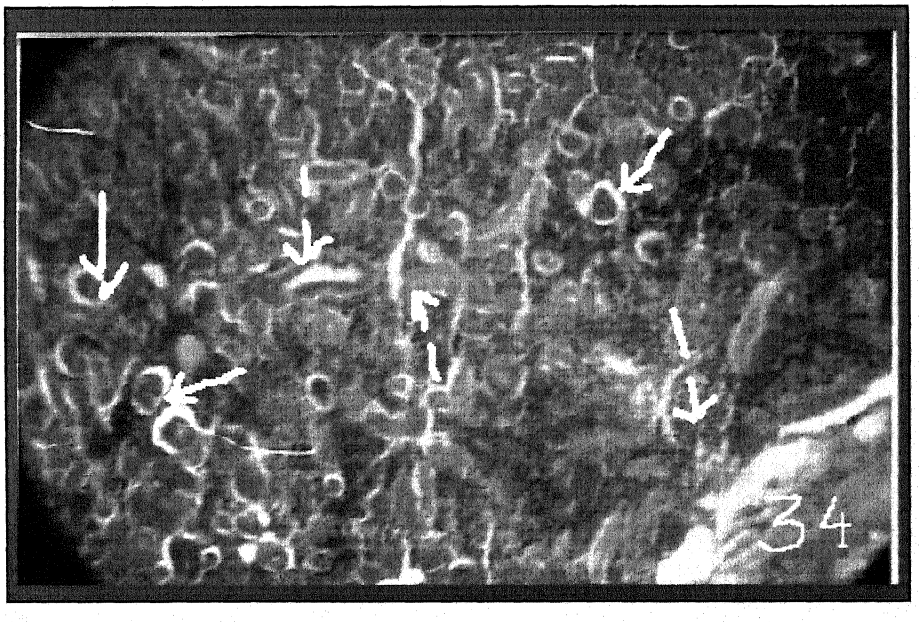
P31 T.S. of kidney of *Labeo rohita* after 10 days exposure to 400.0 µg /l mercuric chloride showing necrosis of epithelial cell (arrow) and fragmentation of haemopoietic tissue (broken arrow) H&E x 400



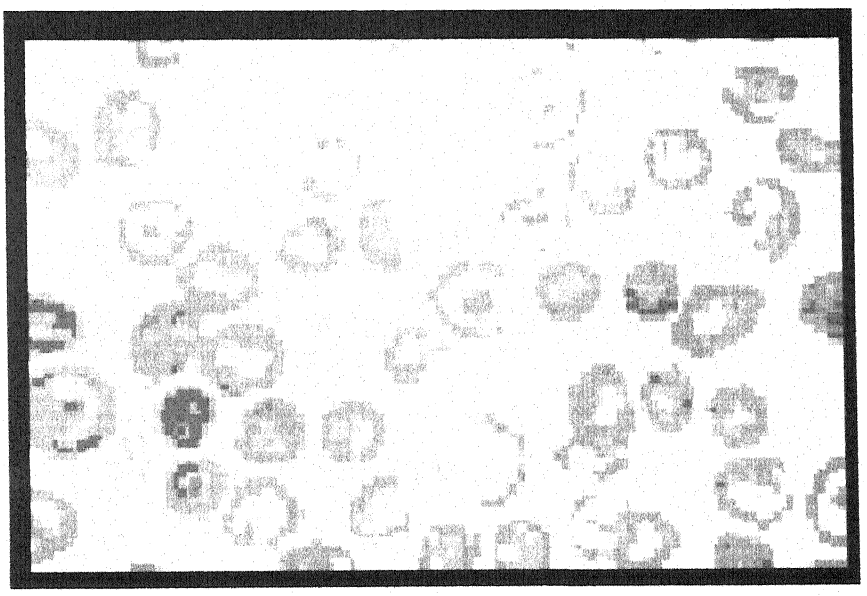
P32 T.S. of kidney of *Clarias batrachus* after 5 days exposure to 2.0 mg /l nickel sulphate showing vacuolar degeneration (arrow) of epithelial cell and haemorrhage (broken arrow) H&E x 400



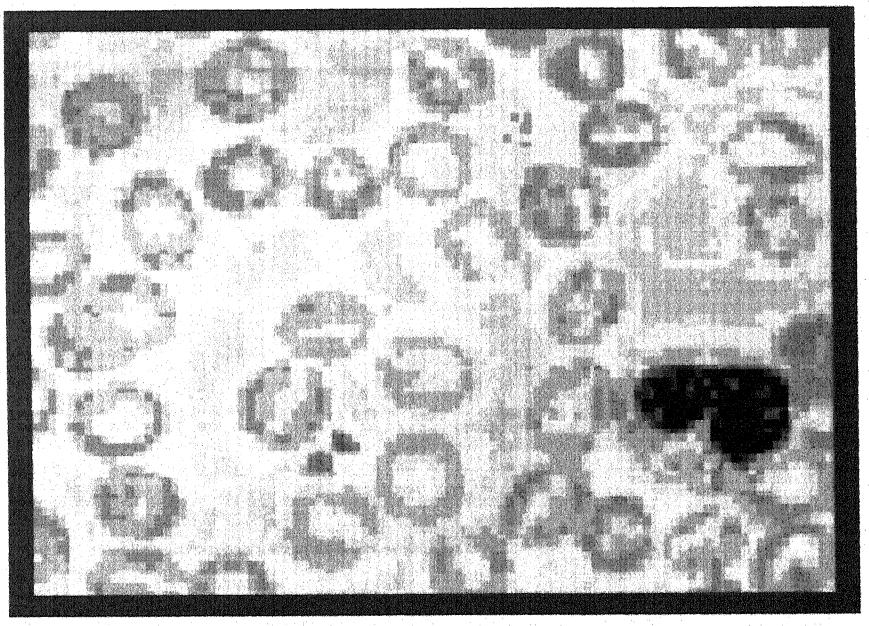
P33 T.S. of kidney of *Clarias batrachus* after 10 days exposure to 4.0 mg/l nickel sulphate showing necrosis of epithelial (arrow) and degeneration of haemopoietic tissue (broken arrow) H&E x 400



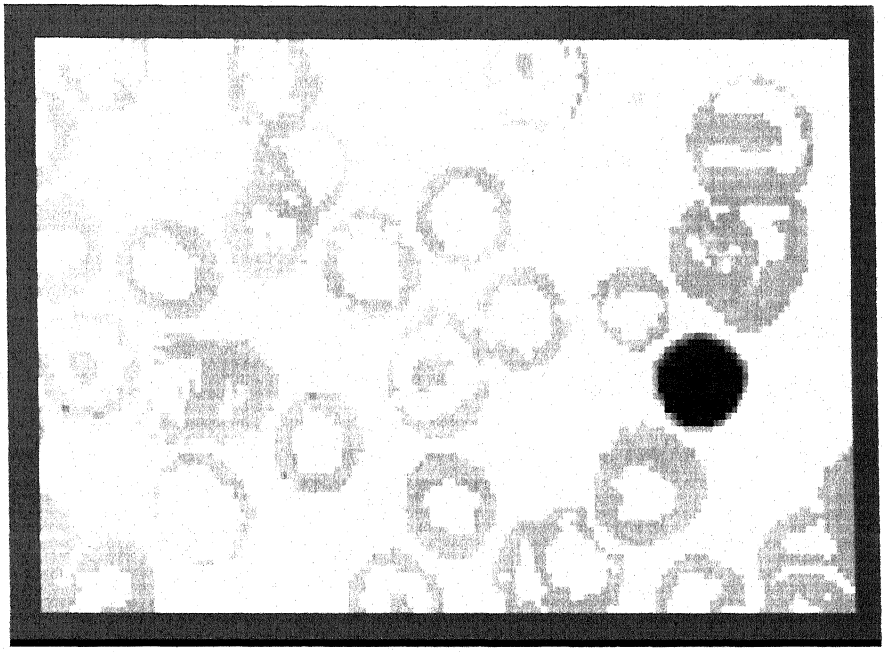
P34 T.S. of kidney of *Clarias batrachus* after 10 days exposure to 8.0 mg/l nickel sulphate showing degenerative change in epithelial cell of tubule (arrow) and haemopoietic tissue (broken arrow) H&E x 400



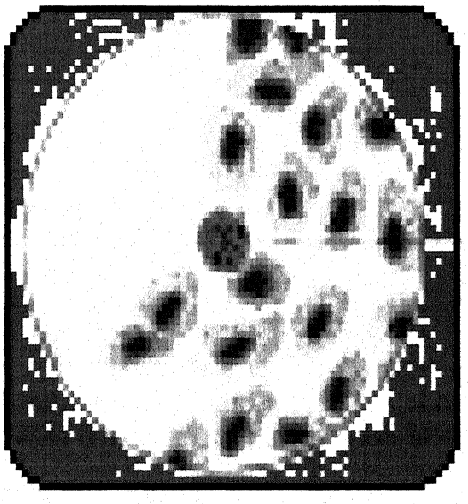
P35 Increased number of Erythrocyte of *Clarias batrachus* after 10 days exposure to 4.0 mg/l nickel sulphate .



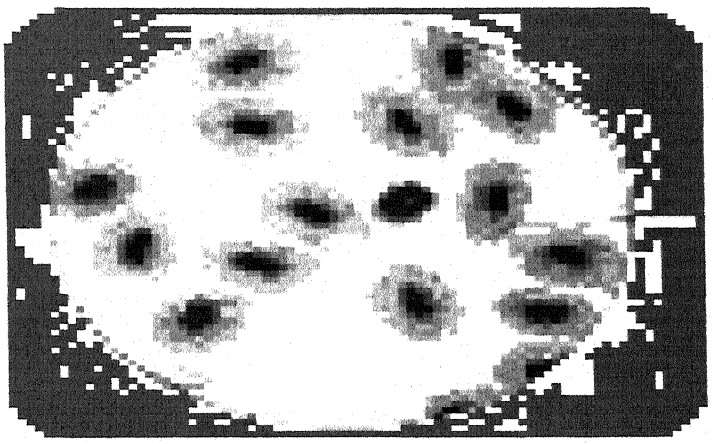
P36 Decreased numberof Erythrocyte of *Clarias batrachus* after 30 days exposure to 4.0 mg/l nickel sulphate .



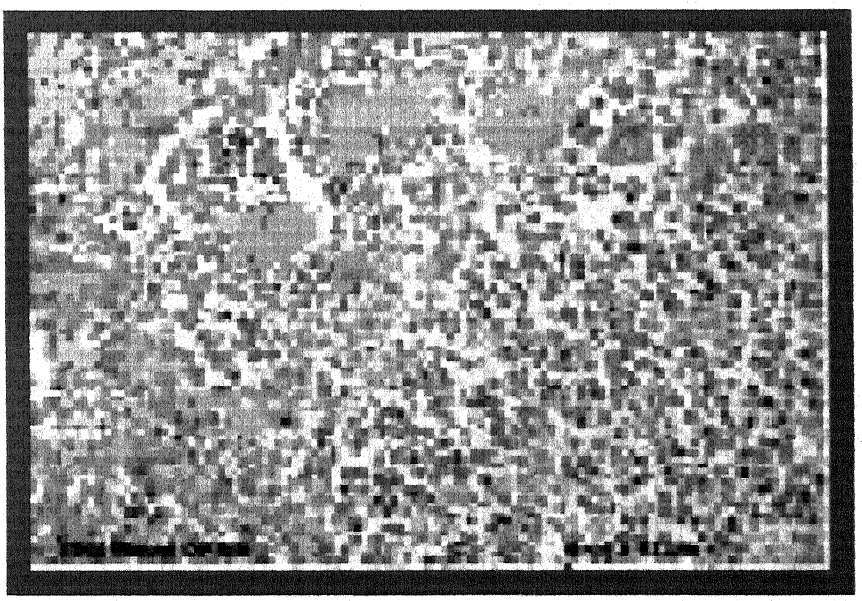
P37 Decreased number of Erythrocyte of *Clarias batrachus* after 30 days exposure to 5.0 mg/l cadmium chloride .



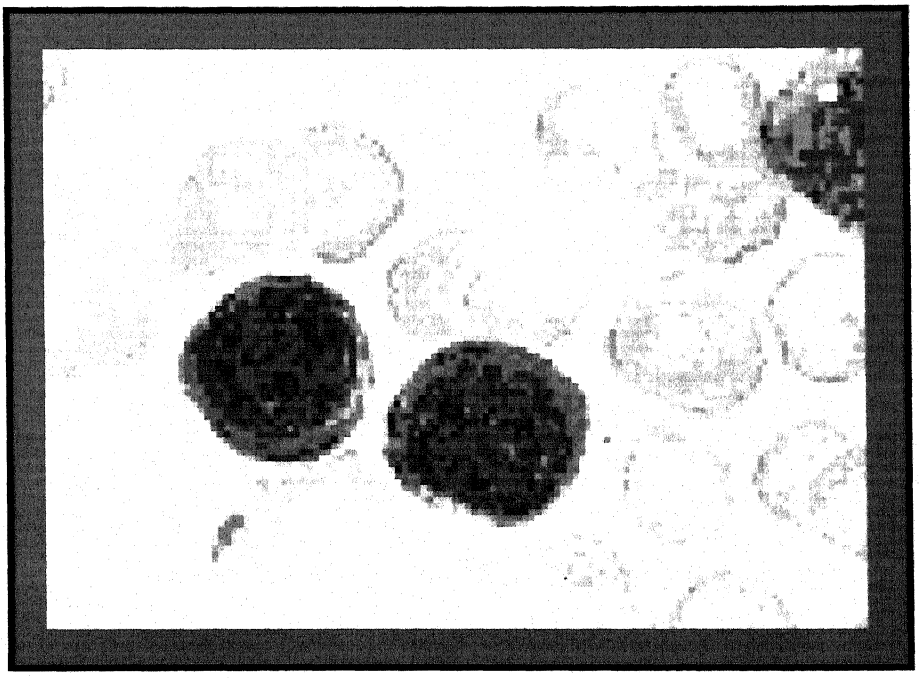
P38 Decreased number of of Lymphocyte of *Clarias batrachus* after 30 days exposure to 5.0 mg/l cadmium chloride .



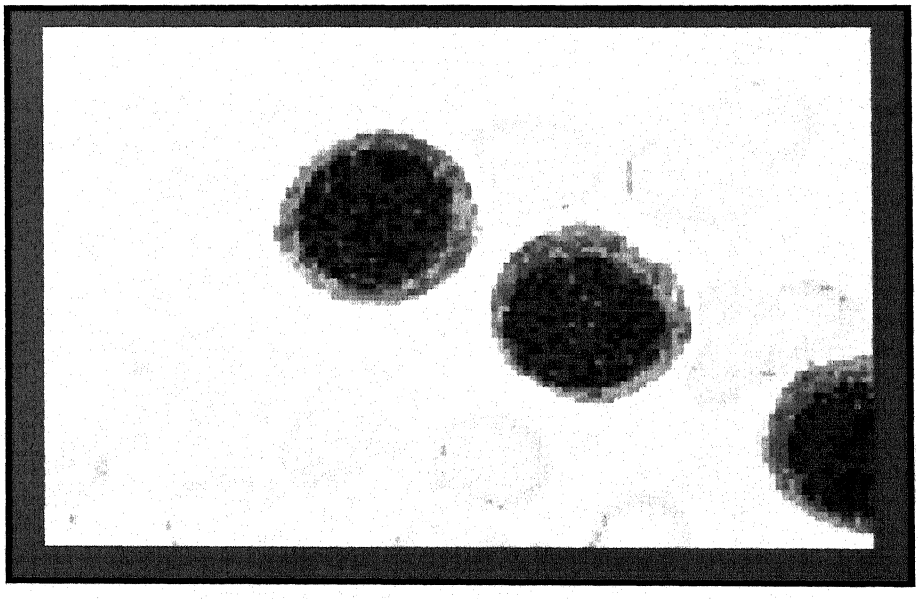
P39 Decreased number of of Lymphocyte of *Clarias batrachus* after 30 days exposure to 4.0 mg/l Nickel sulphate .



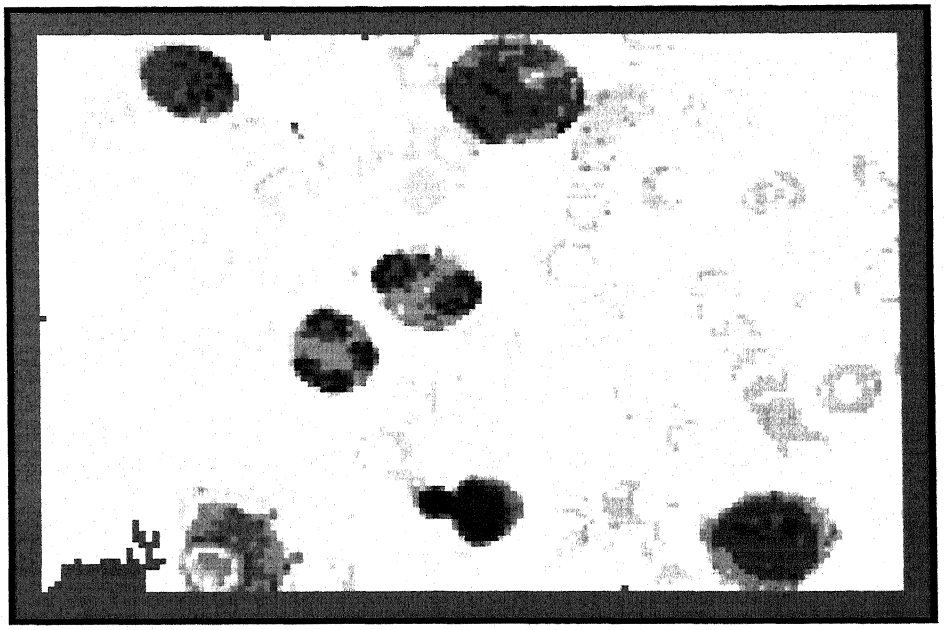
P40 Decreased number of Lymphocyte of *Labeo rohita* after 30 days exposure to 400.0 mμ/l Mercuric chloride showing.



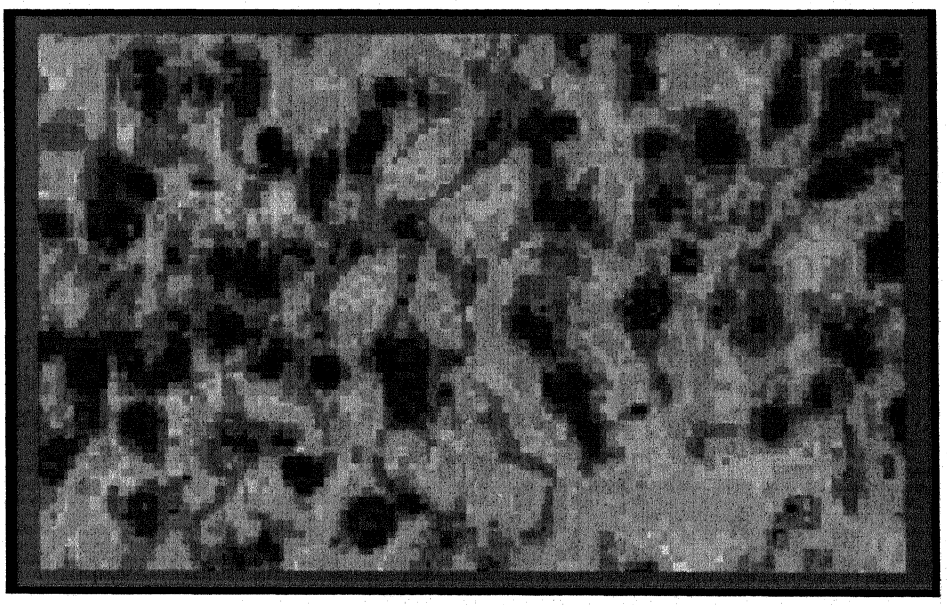
P41 Increased number of Basophils of *Clarias batrachus* after 10 days exposure to 5.0 mg/l cadmium chloride .



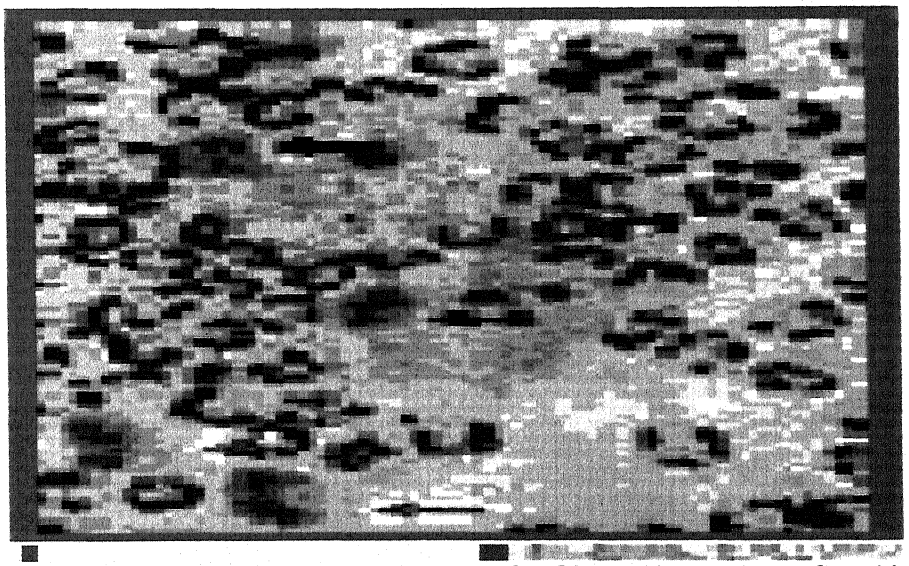
P42 Increased number of Basophils of *Clarias batrachus* after 30 days exposure to 5.0 mg/l cadmium chloride .



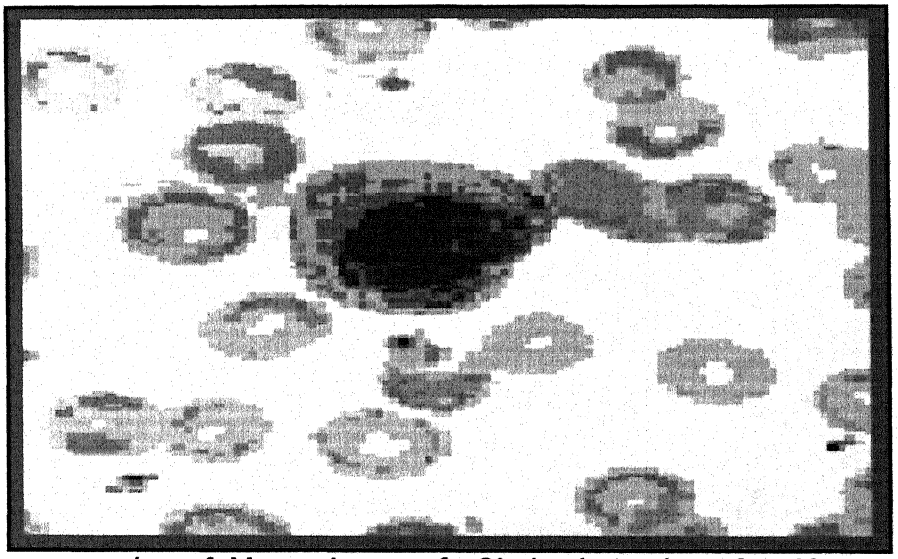
P43 Increased number of Basophils of *Labeo rohita* after 30 days exposure to 400.0 mμ/l Mercuric chloride .



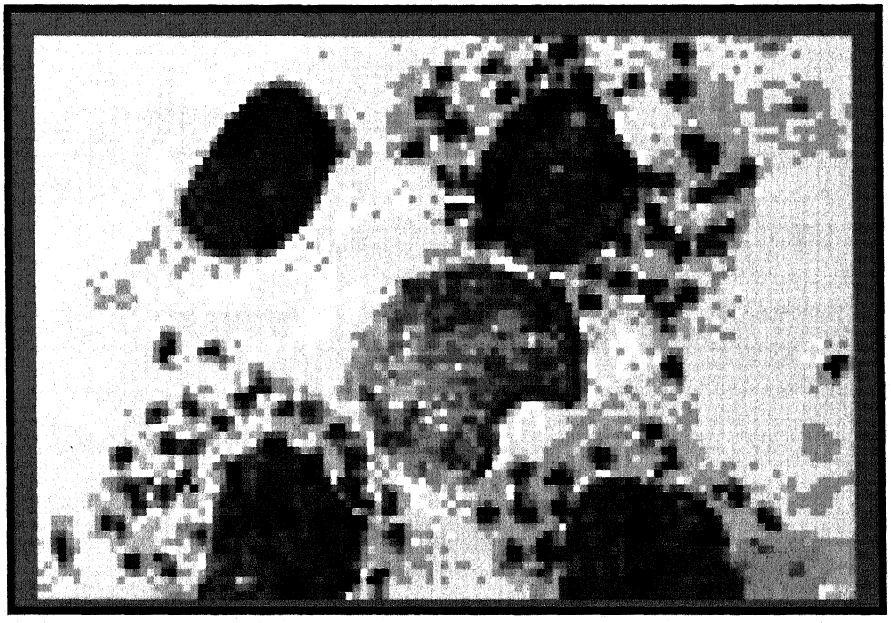
P44 Increased Number of Macrophages of *Clarias batrachus* after 30 days exposure to 5.0 mg/l cadmium chloride .



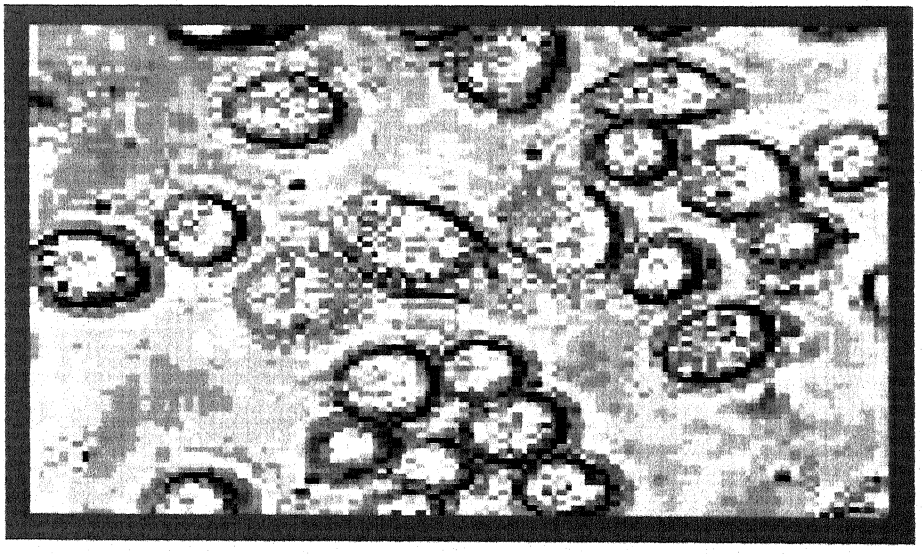
P45 Increased Number of Macrophages of *Clarias batrachus* after 10 days exposure to 5.0 mg/l cadmium chloride .



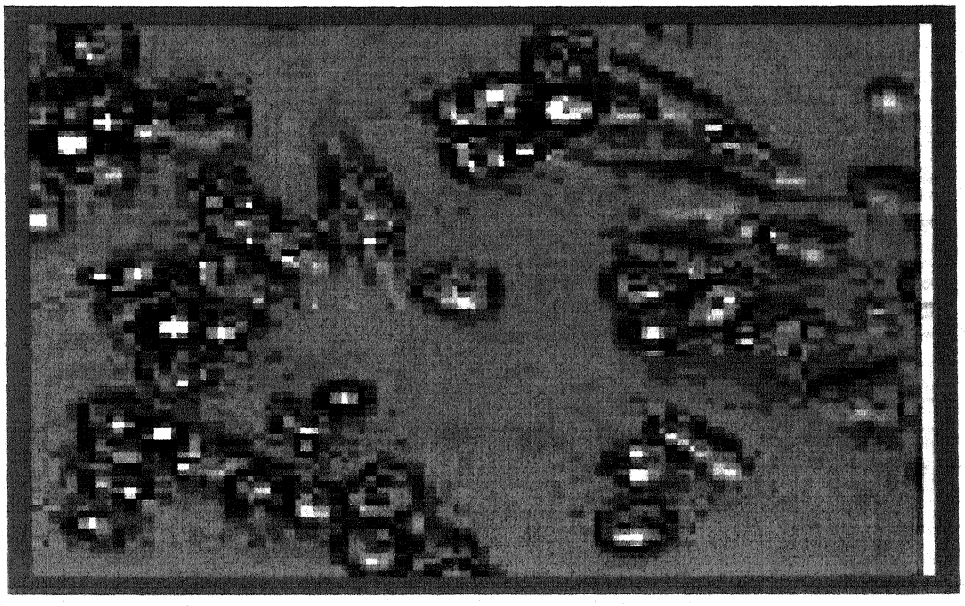
P46 Increase number of Macrophages of *Clarias batrachus* after 10 days exposure to 4.0 mg/l Nickel sulphate .



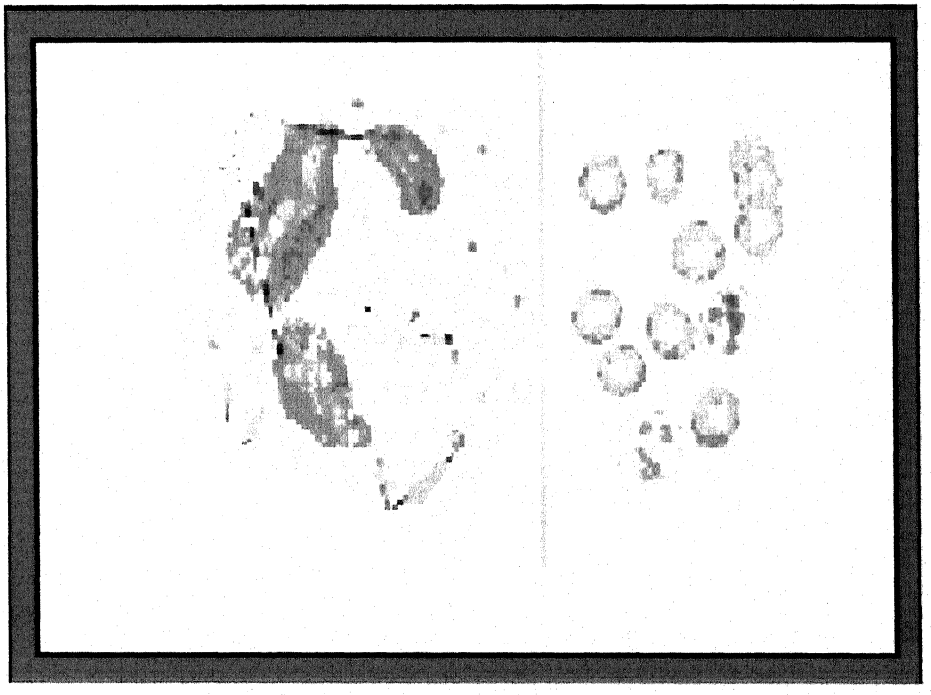
P47 Increase number of Macrophages of *Clarias batrachus* after 30 days exposure to 4.0 mg/l Nickel sulphate .



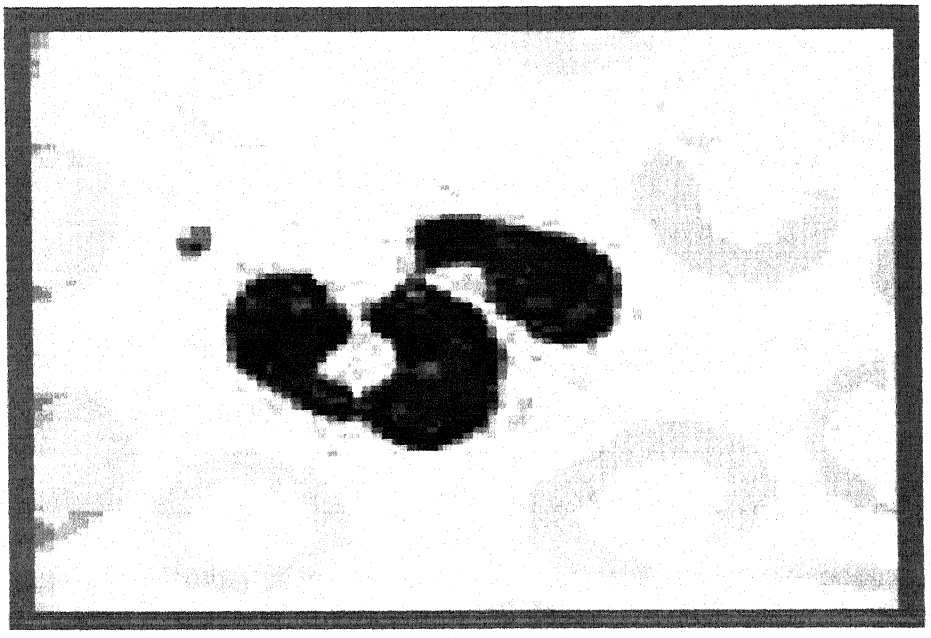
P48 Increase of Macrophages of *Labeo rohita* after 10days exposure to 400 mμ/l mercuric choride showing.



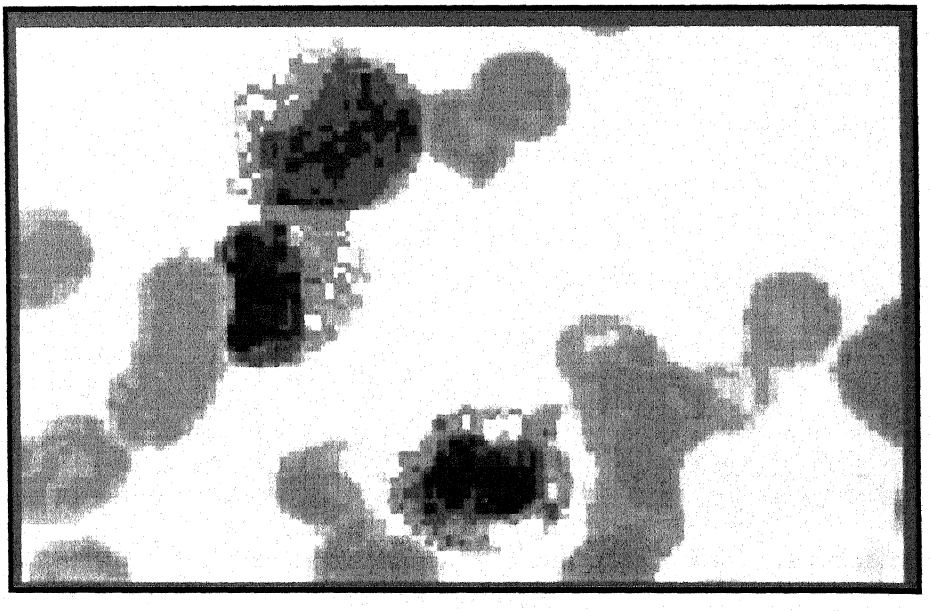
P49 Increase of Macrophages of *Labeo rohita* after 30 days exposure to 400 mμ/l mercuric choride showing.



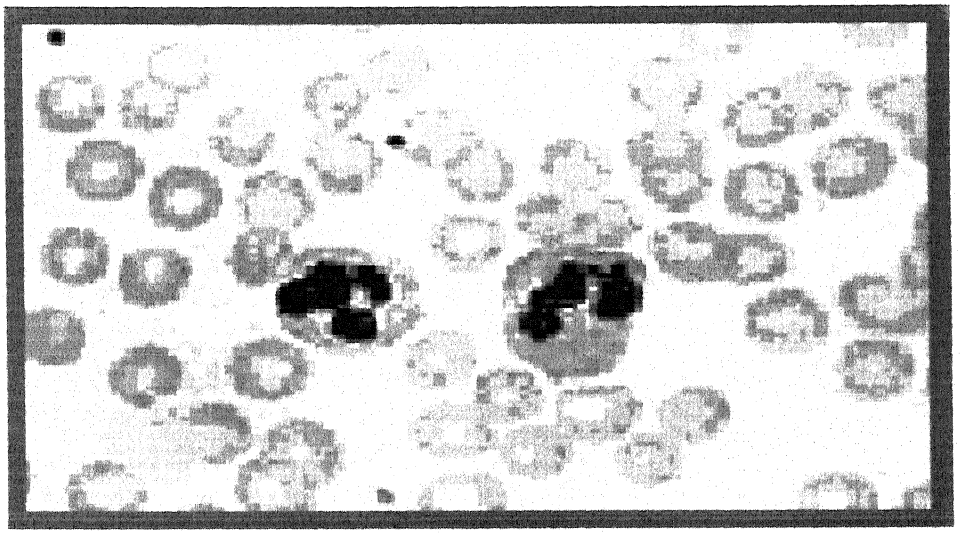
P 50 Number of neutrophils in control Clarias batrachus.



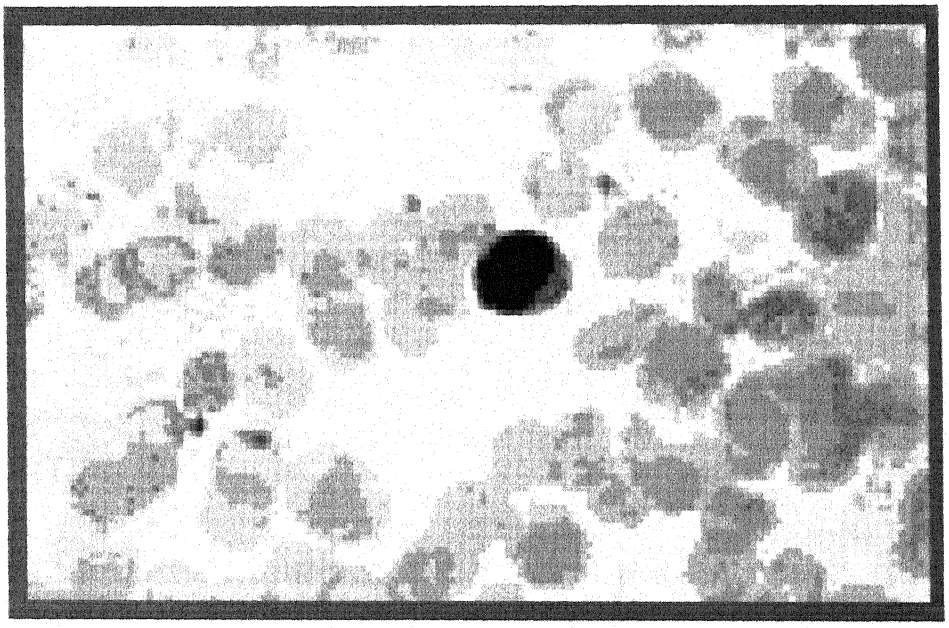
P 51 Decreased number of neutrophils in *Clarias batrachus* when exposure to 5 mg/l Cadmium chloride after 10 days.



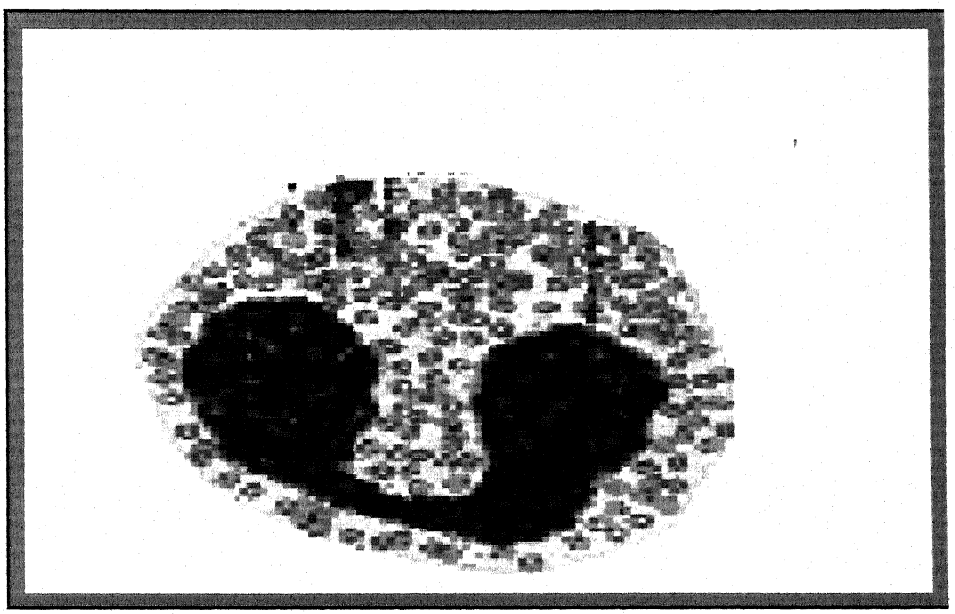
P 52 Decreased number of neutrophils in *Clarias batrachus* when exposure to 5 mg/l Cadmium chloride after 30 days.



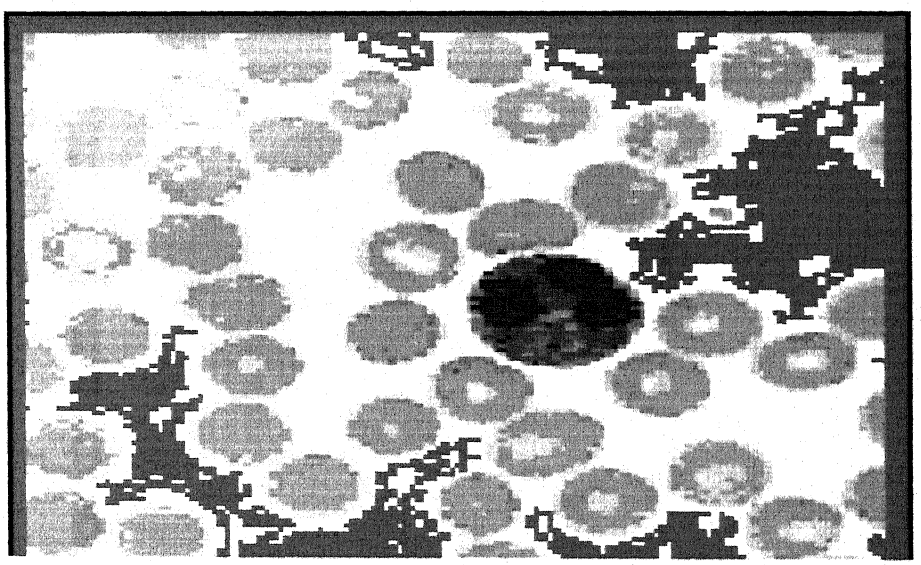
P 53 Decreased number of neutrophils in *Labeo rohita* when exposure to 400 mμ /l Mercuric chloride after 10 days.



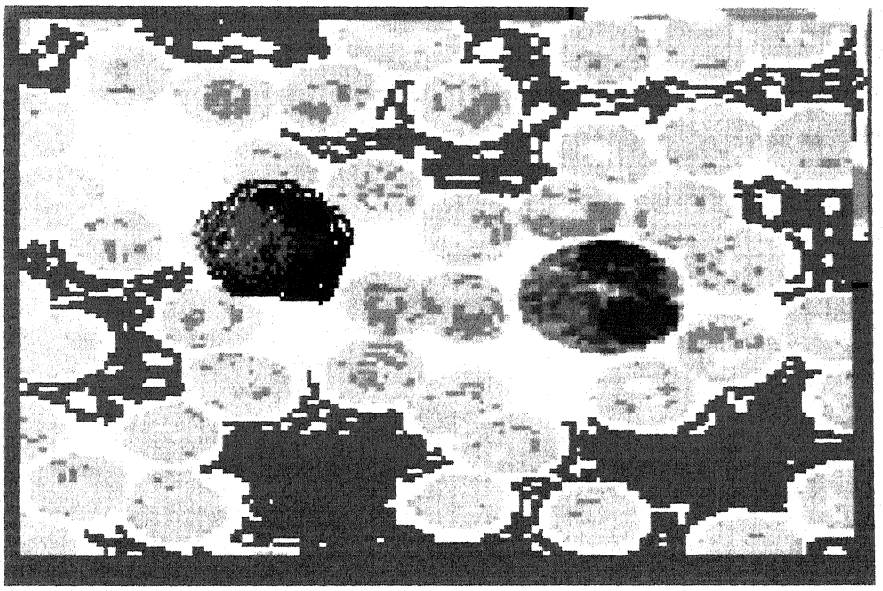
P 54 Decreased number of neutrophils in *Labeo rohita* when exposure to 400 mμ /l Mercuric chloride after30 days.



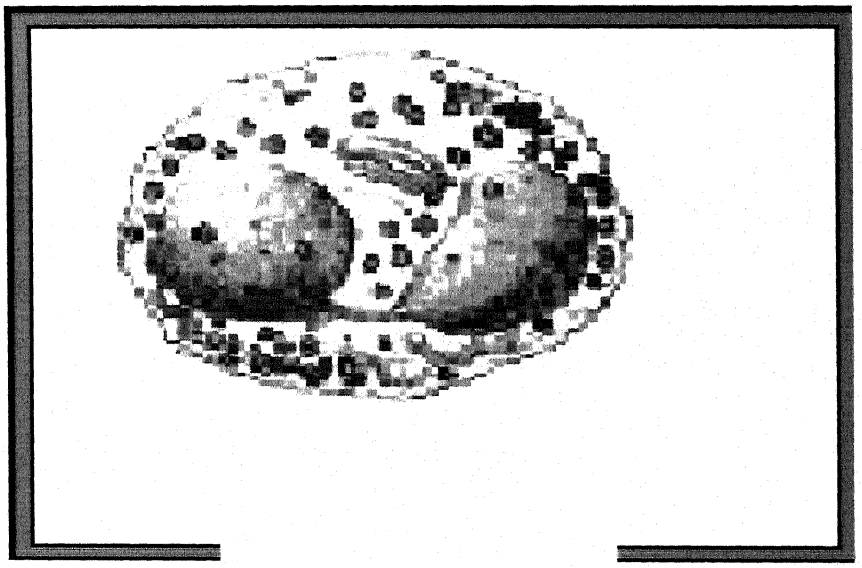
P 55 Eosinophil in control Clarias batrachus



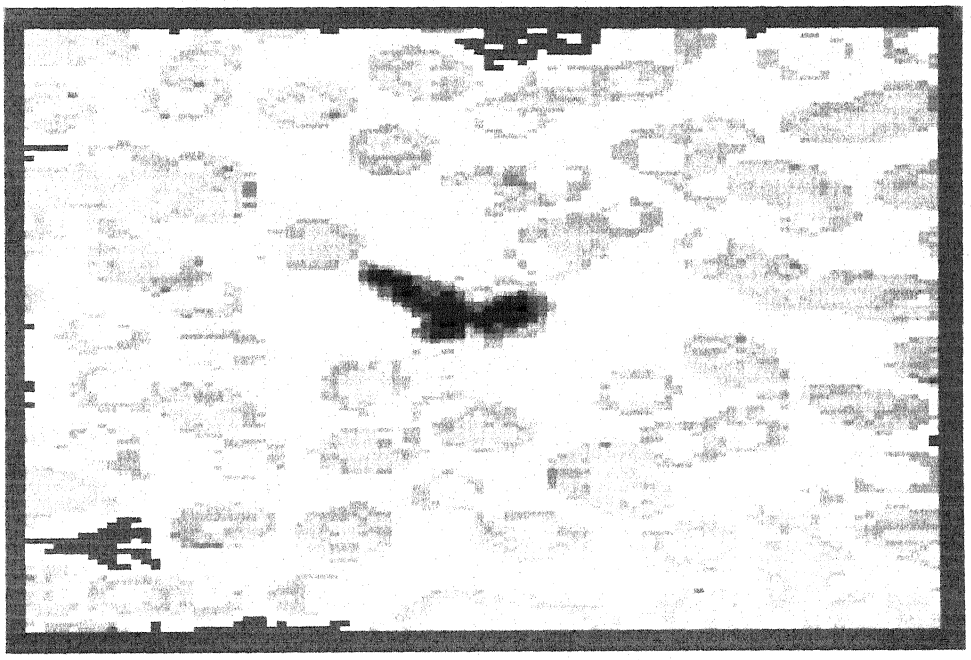
P 56 Increased number of Eosinophils in *Clarias batrachus* when exposed to 5 mg /l Cadmium chloride after10 days.



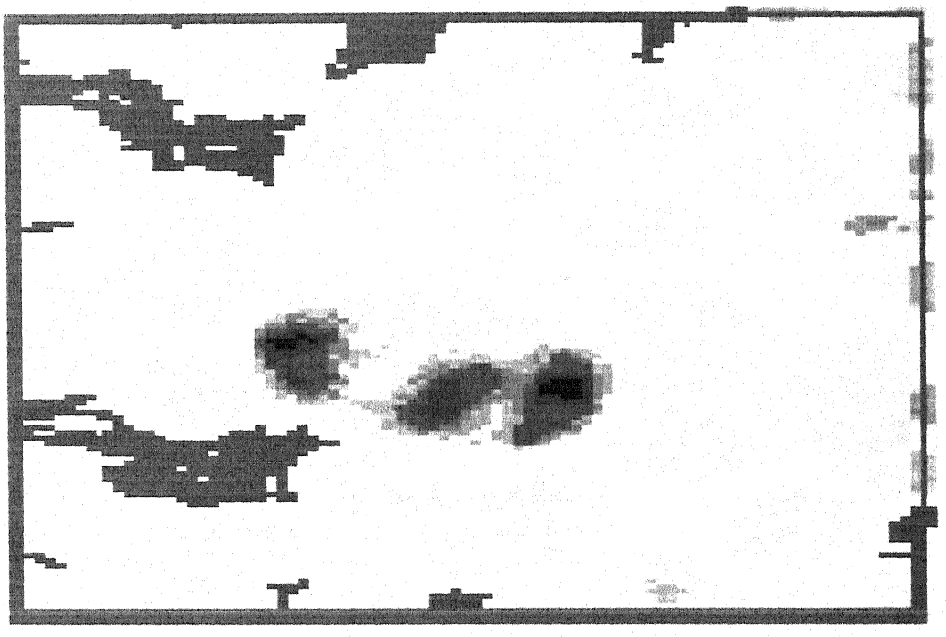
P 57 Increased in number of Eosinophilsz *Clarias batrachus* when exposure to 5 mg /l Cadmium chloride after30 days.



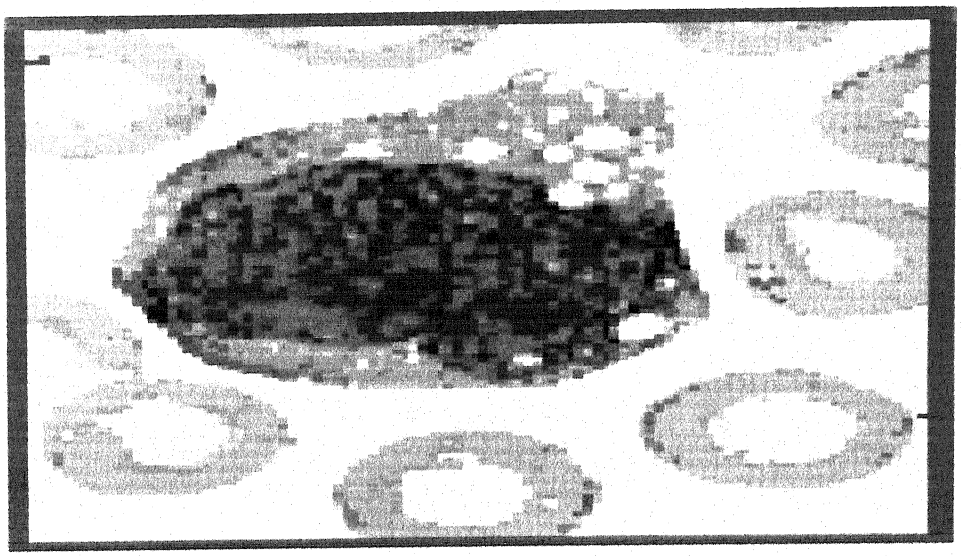
P 58 Eosinophil in control Labeo rohita



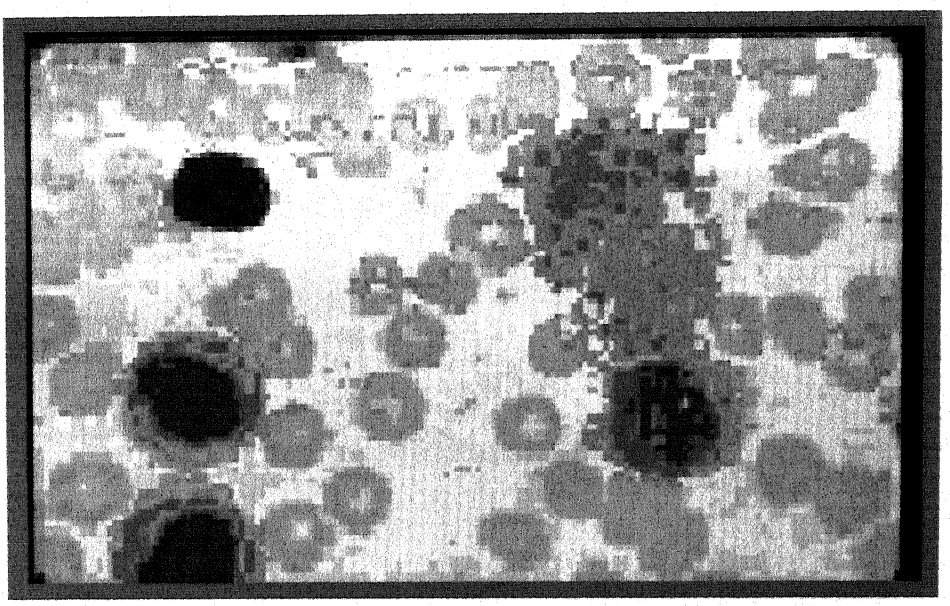
P 59 Increased in number of Eosinophils *Labeo rohita* when exposure to 400 mμ /l mercuric chloride after10 days.



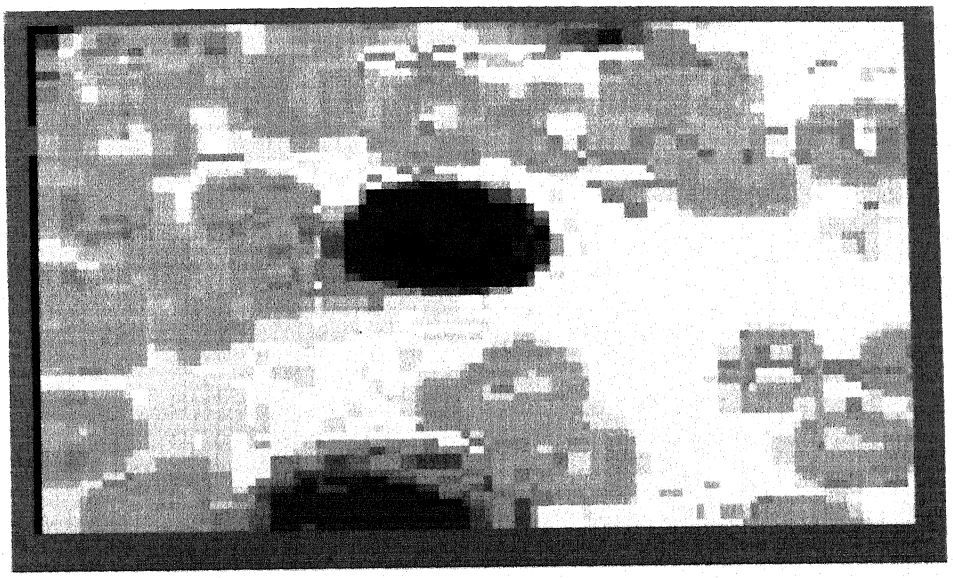
P 60 Increased in number of Eosinophils *Labeo rohita* when exposure to 400 mμ /l mercuric chloride after30 days.



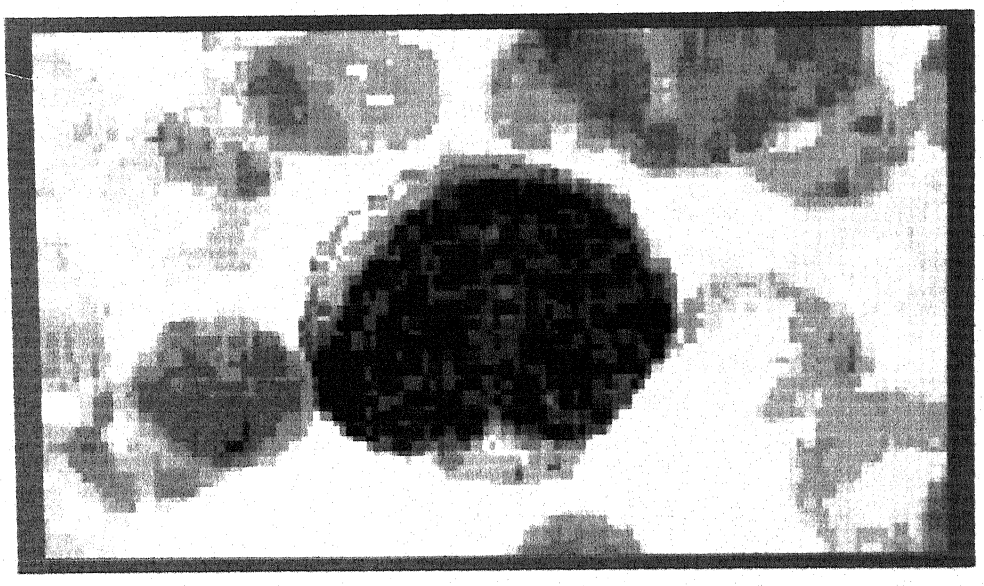
P 61 Monocyte in control *Clarias batrachus*



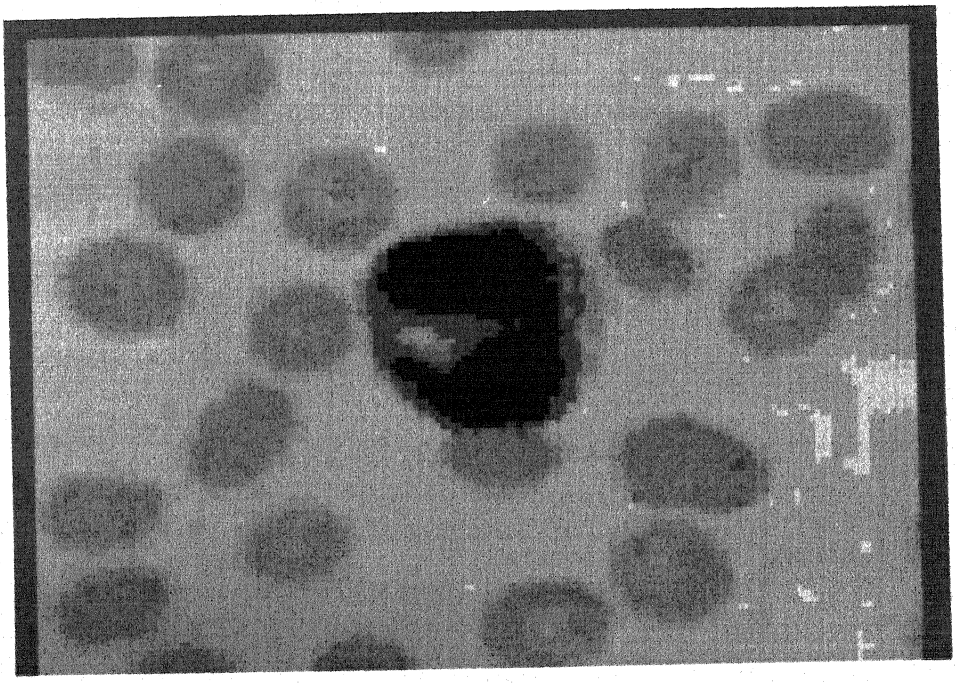
P 62 Decrease in number of Monocytes *Clarias batrachus* when exposure to 5 mg /l Cadmium chloride after10 days.



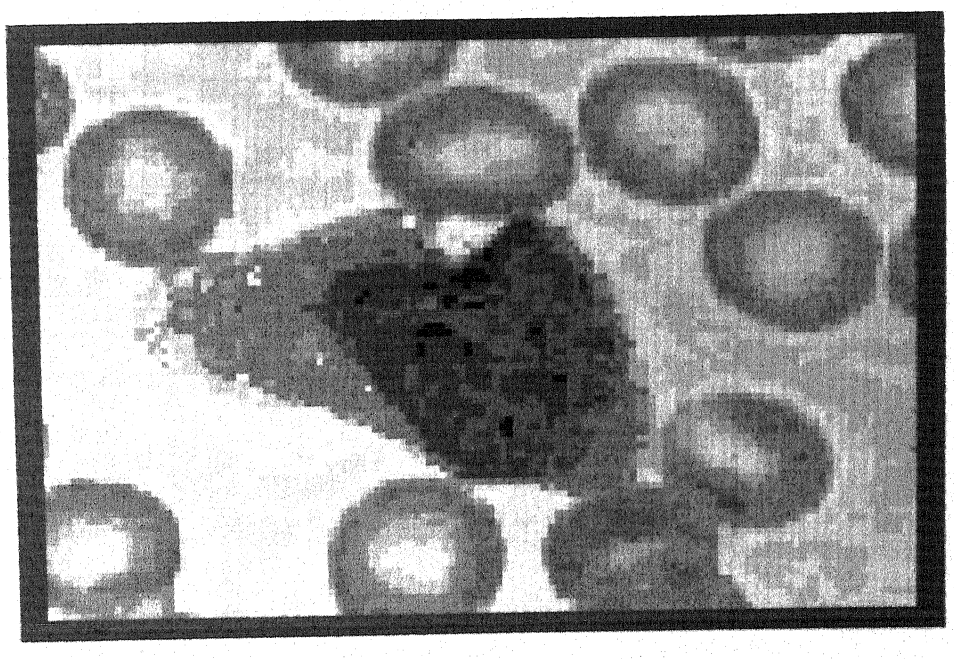
P 63 Decrease in number of Monocytes *Clarias batrachus* when exposure to 5 mg /l Cadmium chloride after30 days.



P 64 Monocyte in control *Labeo rohita*



P 65 Decreased in number of Monocytes *Labeo rohita* when exposure to 400 mμ /l mercuric chloride after10 days.



P 66 Decreased in number of Monocytes *Labeo rohita when* exposure to 400 mμ /l mercuric chloride after30 days.